Cardiac Metabolism Measured Noninvasively by Hyperpolarized $^{13}$C MRI

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Pyrurate is included in the energy production of the heart muscle and is metabolized into lactate, alanine, and CO$_2$ in equilibrium with HCO$_3^-$. The aim of this study was to evaluate the feasibility of using $^{13}$C hyperpolarization enhanced MRI to monitor pyruvate metabolism in the heart during an ischemic episode. The left circumflex artery of pigs (4 months, male, 29–34 kg) was occluded for 15 or 45 min followed by 2 h of reperfusion. Pigs were examined by $^{13}$C chemical shift imaging following intravenous injection of 1-$^{13}$C pyruvate. $^{13}$C chemical shift MR imaging was used in order to visualize the local concentrations of the metabolites. After a 15-min occlusion (no infarct) the bicarbonate signal level in the affected area was reduced (25–44%) compared with the normal myocardium. Alanine signal level was normal. After a 45-min occlusion (infarction) the bicarbonate signal was almost absent (0.2–11%) and the alanine signal was reduced (27–51%). Due to image-foiling artifacts the data obtained for lactate were inconclusive. These studies demonstrate that cardiac metabolic imaging with hyperpolarized 1-$^{13}$C-pyruvate is feasible. The changes in concentrations of the metabolites within a minute after injection can be detected and metabolic maps constructed. Magn Reson Med 59:1005–1013, 2008. © 2008 Wiley-Liss, Inc.

Key words: cardiac metabolism; hyperpolarized C-13; metabolic imaging; pyruvate

Myocardial ischemia associated with coronary artery disease can be studied using several clinical imaging modalities such as x-ray angiography, ultrasound (1), positron emission tomography (PET) (2), single photon emission computed tomography (SPECT) (3), MR imaging (4,5), NMR spectroscopy (6–8), and CT (9), with or without administration of contrast agents. These modalities assess different aspects of myocardial function, viability, and infarction.

Because of its ability to depict cardiac anatomy, wall thickness, and motion as well as function (10,11), MRI has been suggested as a “one-stop-shop” method. Administration of gadolinium (Gd)-based contrast media is used in order to visualize perfusion (12,13). Furthermore, delayed enhancement methods (14,15) have been introduced to demonstrate the extension of infarcted myocardium. However, for a method to be clinically accepted it needs to be capable of delineating the morphological area of interest and when performed routinely it also should allow for readily differentiating the abnormality (pathophysiology) from normal findings.

Hyperpolarization has been introduced as a technique to enhance nuclear polarization, thereby increasing the available signal level in MRI (16–18). The hyperpolarization technique using $^{13}$C, incorporated into small molecules (19,20), has produced polarization levels several orders of magnitude higher than thermal equilibrium values. The use of hyperpolarized $^{13}$C substances for MR angiography (21,22), passive catheter tracking in interventional MRI (23), and perfusion (24,25) has been demonstrated. The dynamic nuclear polarization (DNP) method (20) may be used to enhance the signal of the $^{13}$C-nuclei in endogenous substances such as amino acids, urea, and pyruvate (26,27), making it possible to perform in vivo visualization of the injected substances and their metabolites. The chemical shift range of $^{13}$C facilitates step-by-step tracing of metabolic pathways. This capability has been demonstrated with pyruvate (28).

Pyrurate is a substrate at a central crossroad of intermediary metabolism (29,30) leading to energy production as well as formation of lactate, alanine, and CO$_2$ (Fig. 1). Its fate in the heart muscle has been described extensively both under normal conditions (31,32) and during different cardiac diseases (33,34). Pyruvate enters the muscle cells and is metabolized to lactate, alanine, and CO$_2$, of which the latter will promptly be in equilibrium with HCO$_3^-$. The production of alanine and lactate reflects the general metabolic activity of the cell, whereas production of HCO$_3^-$ reflects mitochondrial activity.

The aim of the present study was to demonstrate in vivo that the metabolic steps following intravenous (i.v.) injection of hyperpolarized 1-$^{13}$C-pyruvate (a key entry point into the citric acid cycle or the Krebs cycle) could be followed by MRI and subsequently used as markers of the metabolic state in pig cardiac muscle cells following different ischemic conditions caused by occlusion of the left circumflex artery.

MATERIALS AND METHODS

Imaging Agent

The polarization and subsequent dissolution of the substance (1-$^{13}$C-labeled pyruvate) was performed using a dynamic nuclear polarizer (20). After the polarization process (60 min of microwave irradiation at 1.2 K), the polarized sample was dissolved and neutralized within 2 sec using a heated aqueous buffer. The final injection solution contained 300 mM pyruvate, 100 mM TRIS buffer (equivalent to ~500 mOsm/L), 0.27 mM Na$_2$EDTA, 250 mM sodium ions, and ~50 μM paramagnetic agent. The tem-
perature and pH were \( \approx 30^\circ C \) and 8.2, respectively. The level of polarization was measured immediately after the dissolution using a low-field NMR spectrometer operating at 2 mT resulting in a \(^{13}\)C Larmor frequency of 22 kHz. A volume of 40 mL was extracted from each polarized dose and inserted into the measuring cell of the spectrometer (21). Throughout the experiments the obtained polarization was in the range of 15–20\%. The \( T_1 \) of \(^{13}\)C pyruvate is about 55 sec in the syringe before injection and, since the transfer time to the imaging magnet was 15–20 sec, the polarization level was estimated to be 10–15\% at the time of administration. The in vivo \( T_1 \) of pyruvate measured in separate experiments was about 25 sec.

Animals

Ten Swedish domestic pigs (4 months, male, 29–34 kg) were examined. The animals were intramuscularly injected with 10 mL ketamine (50 mg/mL; Pfizer, Groton, CT) and 1.5 mL midazolam (5 mg/mL; Pharma Hameln, Germany). The pigs were then intubated and two needles were inserted: one in the hindleg for administration of anesthetic agents and a second one in the front leg for hydration with Ringer’s solution. The second needle was also used for administration of \(^{13}\)C pyruvate and Gd contrast medium. Full anesthesia was induced by using injection of 0.5 mL/kg tiopentalnatrium (Electra-Box, Pharma) and maintained by an infusion (0.6–0.8 mL/min) of ketamine (50 mg/mL; Pfizer), vecuronbromide (2 mg/mL, Organon, West Orange, NJ), and midazolam (5 mg/mL; Pharma Hameln).

The animals were connected to a volume-controlled respirator (PV 301, Breas Medical AB, Sweden, 6-8 mL/kg, 20 breaths per min). Blood \( pO_2 \), \( pCO_2 \), and pH were monitored. The arterial blood pressure was continuously recorded utilizing a catheter placed in the right femoral artery. The heart rate was monitored via MR-compatible ECG-leads. The body temperature was assessed with a nasal probe. Blood levels of glucose and creatine kinase isoenzyme MB (CKMB) were recorded every 30 min. The glucose level was kept constant by adding glucose to the Ringer’s infusion, as required.

After the MRI examinations the pigs were immediately sacrificed. The study was approved by the local ethics committee (Malmö/Lunds djurförsöksnämnd; Application No. M6-05).

Study Protocol

In order to visualize the metabolic patterns induced by different durations of ischemia the animals were divided into two groups of five pigs. Ischemia in the myocardium was induced under x-ray fluoroscopic guidance using a balloon catheter (OB15/2/65, Medi-Tech, Boston Scientific, Watertown, MA) introduced into the left circumflex artery (Fig. 2) via a femoral artery. The occlusion balloon was inflated for 15 min for the first group of animals (stunned group) and for 45 min for the second group (infarcted group), after which the catheter was removed. The myocardium was thereafter reperfused for 2 hr.

Two MR imaging sessions were utilized, one before the induction of myocardial ischemia and another beginning 2 hr after the end of the ischemic procedure. During the first imaging session a chemical shift imaging (CSI) examination was performed following the injection of hyperpolarized \(^{13}\)C-pyruvate in order to assess the metabolic status of the pig myocardium. A multislice retrogated \(^{1}H\) imaging (CINE) examination was also performed in order to visualize the myocardial wall motion and to allow for calculation of the ejection fraction. During the second imaging session the CSI and the CINE examinations were repeated and followed by an i.v. injection of gadodiamide (0.05 mmol/kg; Omniscan, GE Healthcare, Buckinghamshire, UK). For semiquantitative perfusion mapping (12,13) the Gd-bolus was imaged during the first passage through the myocardium. Immediately following perfusion imaging a second Gd injection (0.15 mmol/kg) was administrated. After a waiting period of 15 min the de-


The alanine frequency (TR/TE 90/1.4 ms, FA 10°, slice thickness 7.5 mm/H11003) was used for 13C imaging. The 13C resonance frequency at 1.5T is H11015 MHz. All proton images were acquired using the body coil of the scanner system.

For 13C imaging, shimming was performed at the proton frequency using the previously determined shim values. The 13C transmit/receive coil (Rapid Biomedical, Würzburg, Germany) was used for 13C imaging. The 13C resonance frequency at 1.5T is \( \approx 16 \) MHz. All proton images were acquired using the body coil of the scanner system. For 13C imaging, shimming was performed at the proton frequency using the previously determined shim values. A series of triggered \(^1\)H localizers acquired using a fully balanced Steady-State Free Procession (bSSFP) pulse sequence was used for positioning.

Five minutes prior to each 13C CSI image acquisition multislice retrogated \(^1\)H images (CINE) were acquired in a short-axis view covering the entire heart with a bSSFP pulse sequence (TR/TE 29.7/1.3 ms, flip angle \( [FA] 55° \), slice thickness 8 mm, field of view \([FOV]\) 288 \( \times \) 288 mm\(^2\), matrix 192 \( \times \) 192).

The single slice 13C chemical shift images were acquired in a short-axis view midway through the long axis of the left ventricle (Fig. 3) with the transmitter set on the 13C alanine frequency (TR/TE 90/1.4 ms, FA 10°, slice thickness 20 mm, FOV 120 \( \times \) 120 mm\(^2\), sampled matrix 16 \( \times \) 16, interpolated during reconstruction to 32 \( \times \) 32, pixel size 7.5 \( \times \) 7.5 mm\(^2\), bandwidth 4000 Hz, free induction decay \([FID]\) sampling points 256, FID sampling duration 64 ms). The sequence employed an elliptical central k-space sampling order (35). This is the centric view-ordering scheme that is least sensitive to motion. Compared to sequential view ordering, this view ordering scheme gives the best SNR with respect to the k-space filtering caused by the diminishing transversal magnetization. This sampling schema also increases the line width of the point-spread function (PSF) and may result in image blurring artifacts. The k-space sampling was limited to an elliptically restricted central region of k-space instead of complete 16 \( \times \) 16-sampling, which reduces the total scan time, due to the reduced number of phase encoding steps from 256 to 149, further increasing the line width of the PSF. The sequence was nontriggered, with a total scan time of 13.4 sec.

The Gd perfusion mapping was obtained using a gradient echo pulse sequence (TR/TE/TI 300/1.3/150 ms, FA 15°, slice thickness 10 mm, FOV 192 \( \times \) 192 mm\(^2\), matrix 64 \( \times \) 64, pixel size 3.0 \( \times \) 3.0 mm\(^2\)) using saturation-recovery preparation and the Gd delayed enhancement images were generated using a segmented gradient echo pulse sequence with inversion-recovery preparation (TR/TE/TI 520/4.3/300 ms, FA 30°, averages 2, slice thickness 10 mm, FOV 384 \( \times \) 384 mm\(^2\), matrix 256 \( \times \) 197).

**Image and Spectrum Analysis**

The chemical shift images were calculated using in-house-developed software. The spectroscopic dataset contained one FID from each voxel. The FIDs were exponentially filtered with a signal decay constant of 25 ms and subsequently zero-filled to 1024 points. The corresponding spectra were phase-corrected by manually adjusting constant and linear frequency-dependent phase correction components. A fourth-order polynomial baseline fit (excluding the metabolite peaks) was applied to correct for background distortion around the peaks. The amounts of lactate, alanine, pyruvate, and bicarbonate were quantified by calculating the integral of the respective peaks and are presented as metabolite maps.

In order to quantify the changes in signal, due to the occlusion, the metabolite maps and the pyruvate map were evaluated with the ImageJ software package (36). The left ventricular wall was divided into two areas using the region of interest (ROI) tool. The first area contained the part of the left ventricular wall supplied by the left circumflex artery, and the second area contained the part not supplied by the left circumflex artery, i.e., the rest of the left ventricular wall. The part of the myocardium not supplied by the left circumflex will be referred to as the “healthy region” and the part of the myocardium fed by the left circumflex artery will be referred to as the “diseased region.” For the metabolic maps of the 15-min occlusion (stunned myocardium) subgroup, the boundaries of the diseased region were selected to coincide with the region of decreased metabolism detected in the postischemic bicarbonate metabolite map. For the metabolic maps of the 45-min occlusion (infarcted myocardium) subgroup, the boundaries of the diseased region were selected to coincide with the enhancing region of the proton Gd delayed enhancement. The signals were measured pre- and postocclusion for all ROIs in the metabolite and pyruvate maps for all animals. For pyruvate and for each metabolite the ratio between the signal intensity of diseased and healthy regions were calculated.

In order to perform absolute quantification of the local concentration of the metabolites an external or internal standard may be used. However, due to the limited FOV
and in order to obtain a short scanning time, this was not applied in the present study.

The CINE MR images were visually judged by a radiologist experienced in cardiac MR. They were evaluated using the AHA 17 segment model (37) for localization of wall motion defects. The wall motion defects were graded as normal, hypokinetic, akinetic, or dyskinetic. The radiologist had access to both the preschismic and postischemic CINE image series but was blinded to the occlusion period. The left ventricular ejection fraction was also calculated from both pre- and postischemic CINE images. This calculation was performed using the software package Segment v1.38 (Heiberg Engineering, Höganas, Sweden).

The first-pass Gd images were evaluated on an ROI basis. Pixels of myocardium were separated from pixels containing ventricular blood based on the maximum signal intensity obtained in the image time series. The remaining pixels were divided into six sectors corresponding to the sectors in the mid-slice in the AHA 17 model (37). The maximum upslope of the signal vs. time curve was calculated in each sector, reflecting semiquantitative estimates of the myocardial perfusion (38).

The Gd delayed enhancement images were visually evaluated by the radiologist with the AHA 17 model (37) for localization of enhanced tissue. Enhancement was evaluated by the radiologist with the AHA 17 model (37) for localization of enhanced tissue. Enhancement was judged to be either present or absent.

RESULTS

Blood pressure, heart rate, and ECG were not significantly affected by the pyruvate injections, neither before nor after the ischemic period (data not shown). The pO₂, pCO₂, and glucose levels in blood were kept constant during the experiments. The blood CKMB enzyme levels significantly increased in all five animals exposed to a 45-min occlusion, but no changes were observed in any of the animals after a 15-min occlusion.

Analysis of the wall motion revealed hypokinetic changes in all animals after 15-min occlusion and in three of the five animals exposed to 45-min occlusion. The ejection fraction (EF) did not change significantly in any of the animals after either a 15- or 45-min occlusion.

The Gd upslope perfusion measurement revealed regional changes with a lower perfusion in the infarcted area in two of the five animals exposed to the 45-min occlusion. No changes were detected after a 15-min occlusion. The Gd delayed enhancement was observed in all the animals exposed to 45-min occlusion, but in none after 15-min occlusion. The results from the proton-based methods together with the results from the CKMB test are summarized in Table 1.

The CSI method detected 13C signals from 13C-pyruvate as well as from the 13C-labeled metabolites, lactate, alanine, and bicarbonate in the myocardium of all animals. The typical full-width at half-maximum (FWHM) of the pyruvate peak was ~15 Hz. In all the animals the preschismic images exhibited a homogenous distribution of all three metabolites and pyruvate in the myocardium of the left ventricle (Figs. 4a–d, 5a–d). The highest signal amplitude of pyruvate originating from the nonmetabolized substrate in the blood was detected inside the right and the left ventricle (Figs. 4d, h, 5d, h).

Signal ratios between healthy and diseased areas for alanine, bicarbonate, lactate, and pyruvate, pre- and postocclusion for the 15-min occlusion (stunned) group are shown in Fig. 6. In Fig. 7 the corresponding signal ratios for the 45-min occlusion (infarct) group are shown. The results for bicarbonate, alanine, and lactate are also summarized in Table 2. In the 15-min occlusion group (Figs. 4e–h, 6) the bicarbonate signal in the diseased area was reduced to 41 ± 7.8% compared to the preocclusion value. No significant change in the alanine or the pyruvate signal was detected after occlusion. In the 45-min occlusion group (Figs. 5e–h, 7) the bicarbonate signal and the alanine signal in the diseased area, after the occlusion, were reduced to 6 ± 3.0% and 44 ± 4.9%, respectively, compared to the signal values measured before the occlusion. No significant reduction in the pyruvate signal was detected after occlusion. However, a higher signal was detected in one of the animals. A detailed inspection of the pyruvate map revealed that a folding artifact caused the

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<th>Pig #</th>
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<th>Perf. Post (%)</th>
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The left-hand side of the table shows the results obtained in the 15-min (stunned) group, and the right-hand side shows the results obtained in the 45-min (infarct) group. The ejection fraction is given for pre- and postocclusion. The perfusion values are given as the signal measured in the part of the myocardium influenced by the occlusion relative to the signal in myocardium outside the affected area. In the Late Enhancement column "+" is used to indicate that it was possible to detect an area with enhanced signal due to Gd. In the Wall Motion column "hypo" is used to indicate that the radiologist was able, by comparing pre- and postocclusion CINE images, to detect a hypokinetic motion pattern of the myocardium. In the CKMB column "++" is used to indicate that an elevated creatine kinase isoenzyme MB level was measured.
increased signal. In this animal, due to the limited FOV, the high pyruvate signal in the aorta was folded into the myocardium region. Due to large variance in the measured lactate values, obtained from both groups, no conclusive result could be obtained for changes in the lactate signal. An inspection of the lactate maps confirmed large signal variation artifacts caused by image folding due to lactate formation outside the heart and outside the used FOV.

In Fig. 8 the proton image, showing the slice corresponding to the plane imaged during the $^{13}$C CSI sequences, the Gd-perfusion map, the delayed enhancement image together with the bicarbonate and alanine maps, superimposed on the proton slice, from one of the animals in the 15-min subgroup is shown. The only detected change is a reduction in the bicarbonate signal (Fig. 8e). The corresponding images from one animal in the 45-min subgroup are shown in Fig. 9. The delayed enhancement in the myocardium (Fig. 9c) corresponds to the area showing
58% reduced signal in the alanine map (Fig. 9d) and to the signal void (3%) in the bicarbonate map (Fig. 9e). A small reduction (30%) in the Gd perfusion image (Fig. 9b) was observed.

**DISCUSSION**

Hyperpolarization of molecules conducted in a polarizer outside the MRI scanner produces a polarization several orders of magnitude higher than thermal magnetization of the specimen (16,18). During a hyperpolarization enhanced $^{13}$C imaging experiment, no interfering background signals appear in the images. The detected signal originates exclusively from the hyperpolarized molecules.

Once the polarized substance has left the polarizer, an irreversible decay of the polarization ensues. In the case of $^{13}$C-pyruvate the decay time ($T_1$) is $\approx 55$ sec as long as the substance is kept in a water solution in the syringe. This $T_1$ is within the range reported for other hyperpolarized $^{13}$C substances (21,26,27).

Pyruvate is an endogenous substance that has been proposed as a cardioprotective agent (29,30,39) during ischemic conditions. In these studies doses below 10–18 mmol/kg were considered safe and efficacious. A dose of 10 mmol/kg is 33 times the dose used in the present study. Since hyperpolarization does not change any of the biological or chemical properties of pyruvate, the method should be safe for application in humans, although this needs to be verified carefully for the injection flow rates in question. During clinical implementation of the method employed in the current study the paramagnetic agent, used during the polarization process, should be removed by filtration prior injection of the hyperpolarized substance.

Due to the short in vivo relaxation time of pyruvate ($\approx 25$ sec) no cardiac gating was used during the execution of the $^{13}$C-CSI sequence. The pulsatile motion of the heart resulted in a reduction of spatial resolution. However, this mainly influences the integrity of pyruvate maps and may cause a contamination of signal from inside the heart chambers with signals from the myocardium. The use of an EPI type of readout scheme, where several signal echoes are generated after each excitation pulse, may reduce the scan time and permit implementation of cardiac gating.

In the present study the balloon catheter position in the left circumflex artery was verified by x-ray imaging. The CKMB analysis and the proton MR examinations confirmed effective occlusion of the left circumflex artery. In the 15-min occlusion group the lack of delayed enhancement as well as the lack of CKMB changes confirmed that no necrosis of muscle cells or cell membrane leakage occurred. The hypokinetic wall motion abnormalities confirmed that the muscle cells did not function properly. This phenomena, in which a part of the cardiac muscle wall contracts less than the surrounding normal areas, is called stunning and is thought to be the result of diminished contractile capacity of the cells in that area. In the 45-min occlusion group the rise in CKMB as well as the observation of delayed enhancement confirmed infarction with membrane breakdown and change in extracellular volume.

The ejection fraction was not significantly changed in any of the groups. This may be explained by the relatively small extent of damage to the left ventricular wall and the ability of the heart to compensate for differences in contractility.

The upslope perfusion measurement obtained during the first passage of a Gd bolus also indicated normal perfusion in the damaged area of the heart except in two animals in the infarct group. In these animals the perfusion in the infarcted area was moderately reduced compared to the surrounding tissue. No complete perfusion
obtained in the 45-min (infarcted) group. For the 15-min group the mean signal reduction is 5.8%.

The left-hand side of the table shows the amplitude obtained in the 15-min (stunned) group, and right-hand side shows the amplitude obtained in the 45-min (infarcted) group. For the 15-min group the mean signal reduction is 5.8 ± 8.2% and 56 ± 7.8% for alanine and bicarbonate, respectively. For lactate the mean signal increase is 7.6 ± 31%. For the 45-min group the mean signal reduction is 56 ± 4.9% and 94 ± 3.0%, 14.2 ± 42 for alanine, bicarbonate, and lactate, respectively.

defects were detected, and no reduction was seen in the pyruvate maps, either in the “healthy region” or in the “diseased region,” demonstrating that the hyperpolarized pyruvate reached the infarcted areas in all animals (Figs. 6, 7). This suggests that the reduction of perfusion is here caused by local changes in the small arterioles and capillaries of the myocardium and not by a persistent occlusion or a stenosis of a coronary artery. Since the signal detected from a hyperpolarized nucleus is a linear function of its local concentration, the generated pyruvate maps may be regarded as qualitative perfusion maps. However, a perfusion quantification method using hyperpolarized 13C pyruvate needs to take into account the signal attenuation due to metabolic processes and T1-relaxation together with loss of magnetization caused by RF excitations (25,28).

The production of alanine and lactate in the cardiac muscle after the ischemic period shows that the tissue is still “alive.” Alanine can be produced in different organs in the body, particularly the liver, and transported to the heart. The generated alanine maps do not show any alanine inside either the right or the left ventricle (Figs. 4b,f, 5b,f), confirming that the detected alanine is produced locally in the muscle cells of the left ventricular wall. A part of the lactate may have been generated in the blood cells and lung tissue as indicated by the lactate maps (Figs. 4a,e, 5a,e) where lactate is seen in the larger vessels. The absence of alanine or lactate in the myocardium (Fig. 5e,f) would indicate a serious disturbance in metabolism or cell death. Histological staining with vital dyes may in future studies be used to confirm the absence of infarct in the myocardium (in the stunned group) and to give further insight about myocardium viability.

In the present work the changes in bicarbonate, lactate, and alanine before and after occlusion were measured. However, the results from the measurements of changes in the lactate signal remains inconclusive. The main cause of the large signal variations may be explained by interference from lactate created in the injected bolus before it reached the heart. The lactate present outside the heart, and outside the used FOV, may be folded and superimposed on the myocardium, creating imaging artifacts. Saturation bands placed outside the FOV may be used to suppress this interference, but in the present study this was not applied.

The H13CO3 generated from 13C-pyruvate shows that under normal conditions pyruvate dehydrogenase (PDH) of the mitochondria produces a relatively large amount of 13CO2 which is partly converted into H13CO3 depending on the pH of the tissue. The reduced or absent bicarbonate signal (Figs. 4g, 5g) in the areas where ischemia was present indicated that flux into the citric acid (Krebs) cycle had been diminished. Therefore, metabolic imaging using 13C-labeled pyruvate provides information about: 1) regional perfusion (from the pyruvate map), 2) cellular damage (from the alanine map), and 3) mitochondrial energy production (from the bicarbonate maps). Another important aspect is that the effect on the mitochondria can be detected 2 hr after the occlusion has been resolved. This could be considered a “memory effect” for perfusion deficiencies not resulting in manifest infarcts. As the data (the spectra) collected by this method are quantitative, it should be possible to extend the present method to permit quantification of metabolic flux through glycolytic and TCA cycle pathways (40). However, a quantitative mapping of the flux through the metabolic pathway will demand dynamic CSI imaging methods and would need to take into account differences in relaxation rates in different regions and compartments of the myocardium. By using an external hyperpolarized 13C standard it should be possible to extend the present technique and perform quantification of the local metabolite concentration. However, this would demand the use of a larger FOV, resulting in an increased scan time if the pixel resolution is not to be compromised.

Hyperpolarized 13C-pyruvate facilitates rapid detection of changes in metabolism, distinguishing early metabolic changes in intact cells from more extensive changes associated with cellular breakdown. Longitudinal studies over time can be used not only in the chronic phase, as most other methods do, but also in the acute phase. The method is capable of imaging or evaluating the energy production within the cardiac muscle cells in a 13-sec timeframe. The 13C hyperpolarization technique is compatible with repeated measurements since the hyperpolarized signal following an injection disappears within a few minutes. One possible use of the method could be the diagnosis of patients with unknown chest pain. An infarct would show up on both the alanine, lactate, and bicarbonate maps. As
angina pectoris is caused by a transient ischemic period, no total occlusion is present and pyruvate would reach the cardiac muscle after the episode. No changes would be seen on the alanine and lactate maps, as the uptake into the cells would be normal (no cell death). However, within a certain time after the episode it could be possible to detect the change in mitochondrial activity due to the “memory effect” of the bicarbonate map. If the chest pain was not of cardiac origin, the metabolic maps would be normal. Proton MR imaging, including angiography, would be able to detect other acute sources of chest pain such as pulmonary embolism, pneumothorax, etc. If an infarct was found, it would also be possible to evaluate the size of the infarction as well as the area which could be saved, the so-called “area at risk,” i.e., the area exhibiting reversible changes in metabolism. This would make it possible to select the patients who should be treated actively with thrombolysis or immediate balloon angioplasty. In a more chronic situation the method’s inherent potential for quantification might allow for a better estimate of the amount of cardiac muscle that would benefit from revascularization compared with estimates from MR delayed enhancement images.

Nuclear imaging such as PET imaging (2) with 18fluoro-deoxyglucose can depict the uptake activity of glucose over the cell membrane but cannot differentiate between loss of the cell membranes transport capacity and changes in later stages of the energy generating metabolic processes. However, PET may be used to generate quantitative information. In the procedure used to calculate absolute quantitative heart perfusion maps, one only needs to take into account the half-life of the radionuclide rather than signal decay due to RF pulses or metabolic processes demanded by perfusion methods based on hyperpolarized 13C (24,25,28). Other nuclear medicine methods such as SPECT (3) with Technetium-99 and with the potassium analog Tallium-201 can only depict the perfusion of the heart. These agents may be used to evaluate perfusion and the amount of cell death, respectively, but do not monitor metabolism. Both PET and SPECT methods are time-consuming, model-dependent, insensitive to metabolism (except indirectly by washout of uncharged probes), exhibit relatively low resolution, and come with a nonnegligible exposure of ionizing radiation.

Coronary angiography using either digital subtraction angiography by x-ray (DSA) or contrast-enhanced computed tomography angiography (cCTA) can evaluate the coronary artery morphology. Perfusion and wall motion can also be evaluated by cCTA (8). Neither method can evaluate cell metabolism.

Cardiac MR imaging can depict changes in wall motion (11), perfusion (12), ejection fraction, and also show enhancement in acute infarction as well as in chronic fibrous scars. It cannot distinguish between these last conditions, which might be possible using hyperpolarized 13C-pyruvate imaging. The infarction would show reduced levels of bicarbonate and alanine compared to pyruvate, while the fibrous scar would be expected to show less metabolism of pyruvate. In the present study we were able to detect stunned myocardium in all 15-min occlusion animals. Although the radiologist was blinded to the occlusion time, pre- as well as postocclusion images were available that facilitated the evaluation. In a clinical situation preocclusion images are not generally available, making the evaluation much more difficult. However, in spite of having access to images before and after occlusion, the radiologist did not detect wall motion changes in two out of five animals in the infarction group. One may therefore conclude that the present method may be more sensitive and quantitative for detecting abnormal conditions than standard wall motion techniques.

**CONCLUSION**

Metabolic cardiac chemical shift imaging within 1 min after the injection of hyperpolarized 13C-pyruvate is feasible. The extent of metabolism of this substrate into lactate, alanine, and bicarbonate could be detected and corresponding localized metabolite concentration maps could be produced. The metabolic status as well as the viability of the myocardial cells could be evaluated on the basis of these datasets.

The metabolic consequences of a short coronary artery occlusion without any infarction could be detected 2 hr after the start of reperfusion, demonstrating a “memory effect.” Since pyruvate is essential for energy production in heart muscle, and since this new technique allows for high spatial resolution imaging of the metabolic process within a timeframe of about 10 sec, it should be possible to locally estimate the metabolic rate, or rather the energy consumption rate, in the heart muscle.

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