Nuclear magnetic resonance for the study of cardiac metabolism in diabetes

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Abstract

Magnetic Resonance Spectroscopy is the only non invasive method, not involving exposure to radiation, that allows study of the cardiac metabolism, without the use of a tracer. In the past, most studies of cardiac metabolism were performed with spectroscopy, in different cardiac scenarios (ischemic cardiac diseases, heart failure, for example). This technique should be useful to evaluate and monitor cardiac energy metabolism in diabetes, too. In the future Magnetic Resonance Imaging show promise of becoming a ''one – stop shop'' for studying morphology, function, perfusion and metabolic activity by means of a single imaging technique.
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Nuclear magnetic resonance spectroscopy

Nuclear magnetic resonance (NMR) [1] is an imaging technique that uses the nuclear spin of protons in water and fat to provide anatomical and functional information, for example on the myocardium. Magnetic resonance spectroscopy (MRS) [2] is the only non invasive method, not involving exposure to radiation, that allows study of the metabolic components of the myocardium without the use of a tracer [3].

Proton (^{1}H) NMR is performed immediately before spectroscopy to enable selection of the anatomically most suitable ''voxel'' (the smallest distinguishable box-shaped part of a 3-dimensional space) for the subsequent metabolic study. This aim is achieved by analyzing signals from other nuclei possessing a nuclear spin [sodium-23 (^{23}Na), carbon-13 (^{13}C), phosphorus-31 (^{31}P)], providing information on the chemical and physical state of the tissue.

A current limitation of the technique, confining it to the field of research, is the low sensitivity of the signals acquired from these non proton nuclei. That low sensitivity is secondary to the lower polarization of the nuclei at thermal equilibrium. The level of polarization (P) is defined as:

$$P = \frac{(N^+ - N^-)}{(N^+ + N^-)} \tag{1}$$

where N^+ and N^- are the number of spins in the (parallel) ''up'' and the (antiparallel) ''down'' directions, respectively; the net magnetization, and thus the available NMR signal, is proportional to the ratio of nuclei showing ''up'' spins to that of nuclei showing ''down'' spins. However, despite of the use of a high magnetic field [greater than 3 Tesla (T)] to increase the thermal equilibrium polarization (the energy difference between the two spin states), which contributes to the genesis of a useful signal, the polarization is very low.

Metabolic imaging

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In ^{1}H-MRS, the limit of low sensitivity is overcome by the high concentration of protons in organic tissues, but suboptimal spatial and temporal resolutions limit more widespread use of the technique in the clinical setting: using magnetic fields of 1.5 T, the voxels achieved are large (20–70 ml), compared with 8 ml obtained with 7 T, and acquisition takes about 30–40 min, these lengthy scans being associated with a corresponding increase in motion artifacts. In addition, MRS is technically demanding, and requires specialist expertise and additional magnetic resonance hardware and software [2].

Cardiac metabolism

In the past, most studies of cardiac metabolism were performed with ^{31}P-MRS [4]. This technique mainly examines the ratio between phosphocreatine (PCr) and ATP, which represents a reliable index of the cardiac energy state. During myocardial ischemia, ATP demand exceeds its production, resulting in a decrease in PCr concentrations and, consequently, a decrease in the PCr/ATP ratio [5]. Using ^{1}H-MRS in 10 patients with a history of myocardial infarction, Bottomley and Weiss [6] measured the content of creatine in infarct zones with respect to those in control areas, and found it to be reduced in infarcted areas. With ^{23}Na-MRS it was also possible to show high levels of ^{23}Na signal both in areas of acute necrosis and in chronic myocardial scar tissue. In theory, combination of these three spectroscopic measurements could enable the identification of ischemic, non viable, and hibernating myocardial tissue.

Among these techniques, ^{1}H-MRS is now the easiest to use in the clinical setting because of its higher sensitivity compared with other MRS techniques. A large number of metabolites – including creatine, lactate, carnitine, and lipids – have indeed been studied. The major limitation of ^{1}H-MRS remains the need to suppress the signal deriving from water.

NMR and the diabetic heart

In the research setting, NMR (^{23}Na, ^{13}C, ^{31}P) has provided data on the metabolic changes associated with coronary artery disease, heart failure and its treatment (metabolic therapy with trimetazidine [7]), valvular disease (on a possible future role in the optimal timing for surgical treatment [8]), hypertrophic cardiomyopathy [9], and diabetes mellitus.

Given that diabetic patients surviving a myocardial infarction have higher mortality and higher prevalence of heart failure than non diabetic patients, it would be very useful to evaluate and monitor cardiac energy metabolism in diabetic individuals [10]. Indeed, the cardiac metabolic state has been identified as a major predictor of cardiovascular morbidity and mortality (stronger than left ventricular ejection fraction and New York Heart Association class [11]) in patients with heart failure.

The pathways of myocardial metabolism are summarized in *Figure 1* [12]. In the diabetic heart, glucose and lactate oxidation decrease [13] and fatty acid oxidation is increased [14], increasing the oxygen requirement for ATP production. Studies with MRS in patients with type 1 and with type 2 diabetes have confirmed these observations. For example,
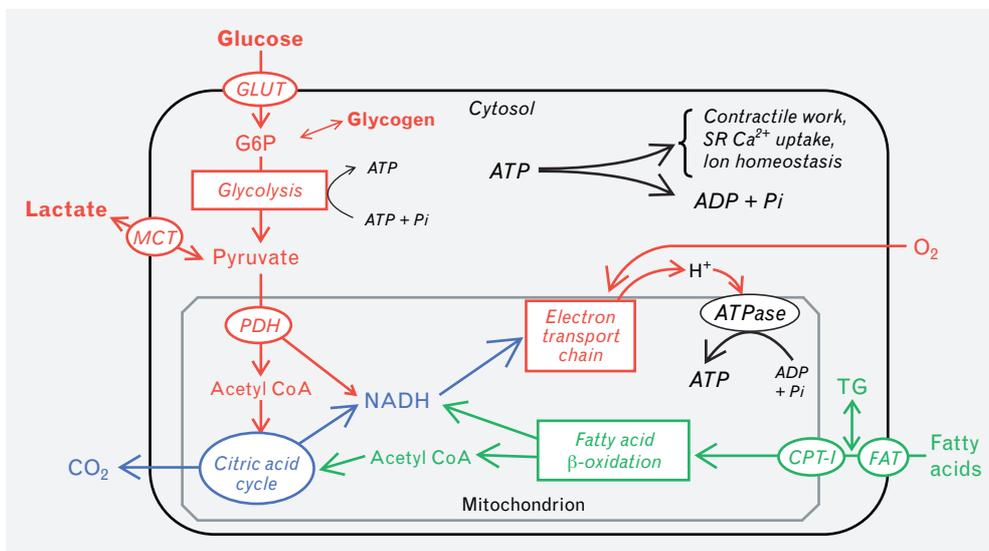

Figure 1. The pathways of myocardial metabolism. CoA, coenzyme A; CPT-1, carnitine palmityltransferase-I; G6P, glucose-6-phosphate; GLUT, glucose transporter; MCT, monocarboxylic acid transporters; PDH, pyruvate dehydrogenase; Pi, inorganic phosphate; SR, sarcoplasmic reticulum; TG, triglyceride. (Modified from Stanley [12].).

Metabolic imaging
Magnetic Resonance Imaging for the study of cardiac metabolism in diabetes
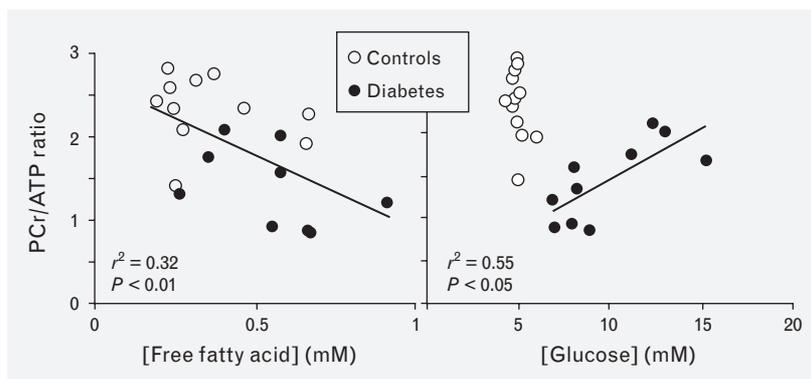

Figure 2. Positive correlation between phosphocreatine (PCr)/ATP ratio and fasting plasma glucose concentrations.

21 patients with type 2 diabetes and normal systolic left ventricular function [normal left ventricular ejection fraction (LVEF)] and normal myocardial left ventricular mass, free from other major cardiovascular risk factors, were studied with ^{31}P-MRS [15]. The PCr/ATP ratio in the diabetes group (1.35) was decreased by 35% relative to that in the control group (2.35), and these figures correlated positively with fasting plasma glucose concentrations and negatively with plasma free fatty acid concentrations (*Figures 2 and 3*). Similar observations (a lower PCr/ATP ratio) were reported in patients with type 1 diabetes and a normal LVEF [16]. These findings are consistent with those from positron emission tomography (PET) studies demonstrating lower rates of uptake of [^{18}F]2-fluoro-2-deoxyglucose [17] and decreased myocardial blood flow [18] in patients with diabetes compared with those in healthy volunteers.

A shift to glycolysis would be more favorable during myocardial ischemia, but this metabolic pathway is less accessible in patients with diabetes. Changes in cardiac substrate metabolism have been identified in the early stage of diabetes, when left ventricular systolic function is fully preserved. Diamant et al [19] suggested that altered energy metabolism (PCr/ATP ratio) could contribute to the left ventricular diastolic dysfunction [evaluated by E/A ratio with magnetic resonance imaging (MRI)] that may occur in patients with type 2 diabetes with normal LVEF.

Previous studies, using Oil Red O staining of explanted hearts at the time of cardiac transplantation, demonstrated cardiac steatosis in diabetic patients with endstage heart failure [20]. Before the publication of the paper by McGavock et al [21], it was unknown if this phenomenon was a cause or a consequence of heart failure. Using ^{1}H-MRS, McGavock and colleagues found an increased myocardial triglyceride content in patients with impaired glucose tolerance and type 2 diabetes in early stage of the disease (*Figure 4*). This study thus demonstrated that cardiac steatosis precedes the onset of diabetes mellitus and left ventricular systolic dysfunction.

The study of myocardial energy metabolism in the diabetic population is a promising application of spectroscopy in the clinical setting. Myocardial spectroscopy could identify the various stages of diabetes and could help towards an understanding of the tight connection between diabetes and cardiovascular diseases, eventually enabling objective assessment of metabolic treatments.
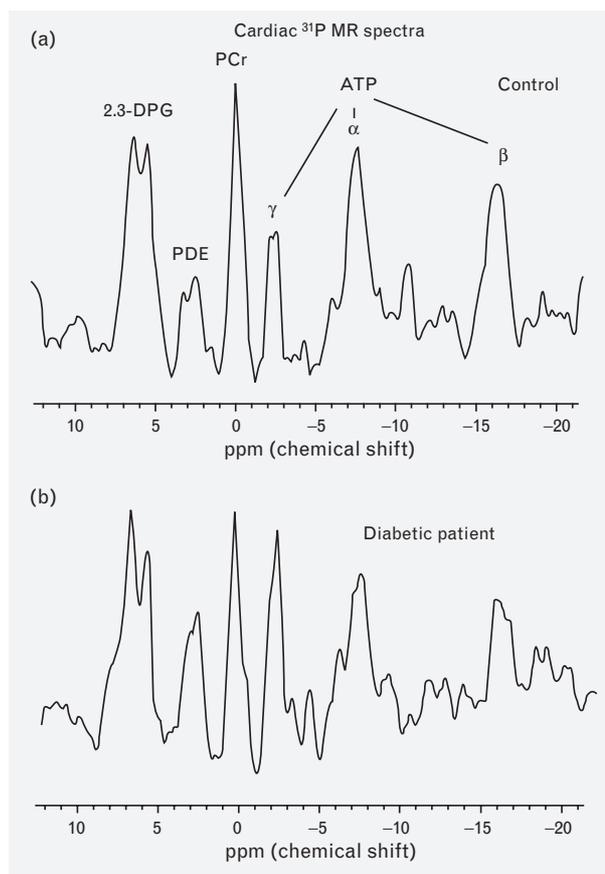

Figure 3. Phosphorus-31 spectra in (a) a normal control individual and (b) a diabetic patient.

Metabolic imaging

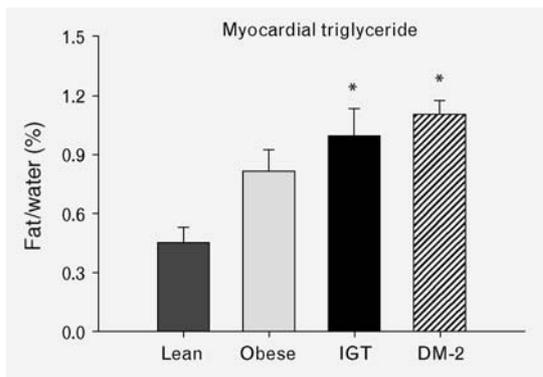*Giancarlo Todiere and Mario Marzilli*

Figure 4. Myocardial triglyceride content, measured by proton magnetic resonance spectroscopy and expressed as fat/water (%), in lean and obese individuals and in patients with impaired glucose tolerance (IGT) or early type 2 diabetes (DM-2). *$P < 0.01$ (Adapted from McGavock [21].).

Figure 5. Schematic diagram of the orientation of spin nuclei at thermal equilibrium and in a hyperpolarized state.

Technological considerations

The use of spectroscopy in the clinical arena strongly depends on technological progress.

The transition from 1.5 T to 3 T MRI has already significantly improved the spatial resolution; further improvements are expected with 7 T magnetic fields, which could provide adequate coverage of the entire heart with a spatial resolution corresponding to a 17-segment model [11]. However, increasing the magnetic field gives rise to problems of cost, depth of penetration of the radiofrequency instrumentation, and the available sequences and tissue contrast.

An alternative strategy with which to improve the signal-to-noise ratio while limiting the increase in the magnetic field with all its attendant negative con-
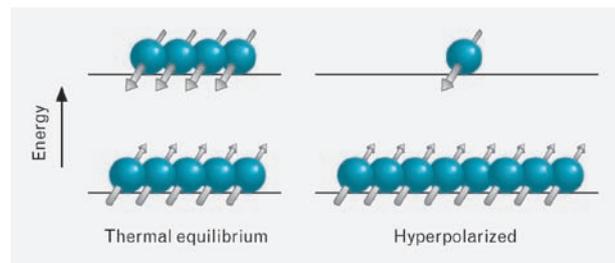
sequences, lies in a different means of increasing the polarization – a ''hyperpolarization'' technique [22]. This method can increase the signal by several orders of magnitude, using magnetic fields of relatively low strength (up to 3 T) (*Figure 5*). Referring to equation (1), and bearing in mind that the signal-to-noise ratio (SNR) is proportional to the gyromagnetic ratio (γ), the concentration of nuclear spin (c) and the polarization (P) ($SNR = c\,\gamma\,P$), it is clear that creating a non equilibrium distribution of the nuclei ($N^+ - N^-$) will achieve an increase in the signal-to-noise ratio. The main advantage of this approach is that the hyperpolarized nuclei create the signal themselves, rather than moderating the signal from surrounding protons.

Carbon-13 is able to label any substance that contains carbon, including glucose, pyruvate, and proteins. Given the absence of ^{13}C in biological tissues, the images are free from background signal, whereas the intensity of the signal is a linear function of its concentration (quantitative perfusion). Therefore, ^{13}C-NMR could be used in studies of cardiac metabolism, playing a role similar to PET but with better spatial resolution and without exposing the
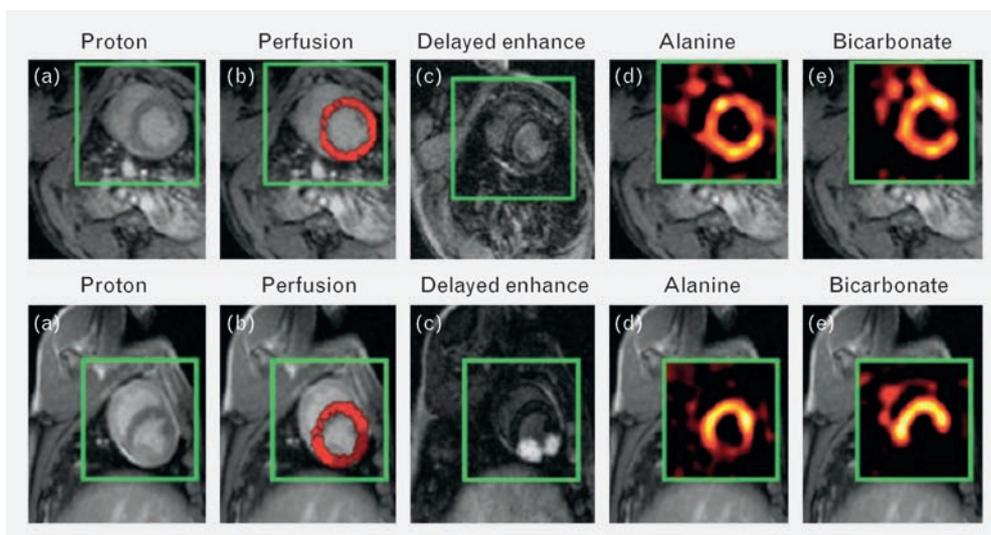

Figure 6. Myocardial images obtained using hyperpolarization-enhanced ^{13}C magnetic resonance imaging after 15 min (top row) and 40 min (bottom row) of occlusion of the circumflex artery. (a) Proton (^{1}H) NMR images. (b) Semiquantitative gadolinium-enhanced perfusion maps. (c) Delayed gadolinium-enhanced images showing myocardial fibrosis. (d, e) Mapping of alanine (d) and bicarbonate (e). (Modified from Golman [23].).

patient to ionizing radiations. PET with fluorodeoxyglucose makes it possible to evaluate glucose uptake by the cell, but, unlike ^{13}C-NMR, cannot discriminate between changes to the mechanisms of cellular transport and intracellular metabolic pathways downstream. The major limitation of this technique is that the hyperpolarization is a short physical phenomenon – its half-life is only several minutes – so MRI laboratory facilities capable of performing the hyperpolarization in situ are essential.

In a recent study, Golman et al [23] combined ^{13}C-NMR and ^{1}H-NMR to examine metabolism in the ischemic heart. Myocardial ischemia was induced by ligation of the circumflex artery for 15 min and myocardial necrosis was induced by an occlusion lasting 45 min. The group estimated myocardial perfusion (using a paramagnetic contrast agent), regional and global function, and myocardial fibrosis (using delayed enhancement); they also quantified metabolites such as pyruvate, alanine, and bicarbonate in normal, ischemic, and necrotic tissue. They found reduced alanine and bicarbonate signal from areas with hyperenhancement (*Figure 6*). Production of lactate and alanine is a reliable indicator of the metabolic activity of the cell, whereas production of bicarbonate reflects mitochondrial activity.

Together, ^{1}H-NMR and ^{13}C-NMR show promise of becoming, in the near future, a ''one-stop shop'' for studying morphology, function, perfusion, and metabolic activity by means of a single imaging technique. ◼

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