

# Evaluating metabolic changes in heart disease by magnetic resonance spectroscopy

Michiel ten Hove and Stefan Neubauer

Department of Cardiovascular Medicine, University of Oxford, Oxford, UK

Correspondence: Dr Michiel ten Hove, Department of Cardiovascular Medicine, Wellcome Trust Centre for Human Genetics, Roosevelt Drive, Oxford OX3 7BN, UK.

Tel: +44 (0)1865 287604; fax: +44 (0)1865 287586; e-mail: michiel.tenhove@well.ox.ac.uk

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### Abstract

Magnetic resonance spectroscopy (MRS) is a versatile and powerful tool for the non invasive study of cardiac metabolism and can be used to measure myocardial concentrations of many different metabolites. The most widely studied nucleus, phosphorus-31, allows for the detection of phosphocreatine, ATP, intracellular pH, and flux through the creatine kinase reaction. Carbon-13-MRS has a low sensitivity, but several metabolites can be measured to facilitate study of substrate utilization and flux. Finally, hydrogen-1 (proton)-MRS can be used to study myocardial oxygenation and creatine concentrations. Here, we give a brief overview of the different applications of cardiac MRS and the pathophysiological insights derived from such studies.

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Magnetic resonance imaging (MRI) has become a routine diagnostic tool in clinical cardiology, but magnetic resonance spectroscopy (MRS) has to date been applied only in research. In contrast to MRI, which uses signal derived from protons ( $^1\text{H}$ ) in water and fat, MRS uses signals from many different nuclei. The major challenge when applying MRS is the intrinsic low resolution. However, MRS can be used to measure concentrations of many different compounds, intracellular pH, substrate selection, and even enzyme kinetics in the intact heart. MRS, like MRI, is truly non invasive and non destructive, and does not require radioactive tracers or harmful ionizing radiation.

Most cardiac MRS studies have been performed on the isolated heart model or in vivo in humans. Using isolated hearts has the advantage that signal from the entire heart is acquired, increasing sensitivity and

making absolute quantification relatively straightforward. In-vivo MRS requires localization techniques and is complicated by the fact that movement necessitates cardiac and, possibly, respiratory gating, all of this inevitably leading to signal loss. Absolute quantification of metabolite concentrations is also difficult when in-vivo MRS is being used. Therefore, results from in-vivo phosphorus-31 ( $^{31}\text{P}$ )-MRS are often presented as phosphocreatine (PCr):ATP ratios, although absolute quantification is preferable where possible.

Phosphorus-31 is the nucleus most widely used to study cardiac metabolism and allows for the detection of phosphocreatine, ATP, inorganic phosphate (Pi), and intracellular pH. ATP is the main substrate for all energy-consuming reactions in the cell, whereas phosphocreatine buffers ATP concentrations and transports energy within the cell via the creatine

kinase reaction:



where Cr is creatine. Flux through this reaction can also be measured with  $^{31}\text{P}$ -MRS.

Magnetic resonance spectroscopy can easily be used in a longitudinal fashion, allowing for monitoring of changes resulting from various pathologies over time in the same heart. Metabolic changes during ischemia and reperfusion have been studied extensively both in isolated hearts and in vivo. During global ischemia, phosphocreatine rapidly decreases to near undetectable concentrations, whereas ATP decreases much more slowly [1], illustrating the buffering capacity of (phospho)creatine. Heart failure has also been extensively studied using MRS. It was shown that, in intact residual myocardium of rat hearts with chronic myocardial infarction, phosphocreatine concentrations and flux through the creatine kinase reaction are both reduced [2]. Similar results were obtained in other animal models of cardiac hypertrophy and failure, such as dogs with volume-overload hypertrophy as a result of severe mitral regurgitation [3] and Syrian cardiomyopathic hamsters with advanced heart failure [4].

Results from studies on human heart failure have confirmed findings in animal models: the myocardial PCr : ATP ratio is reduced in symptomatic patients with dilated cardiomyopathy [5] (Figure 1). This reduction correlates with left ventricular ejection fraction [6]. The myocardial PCr : ATP ratio was even found to be a better predictor of long-term survival of patients with dilated cardiomyopathy than either left ventricular ejection fraction or the New York Heart Association class [7]. Measuring absolute concentrations revealed that, in patients with severe heart failure, ATP concentrations per se are also reduced [8], implying that PCr : ATP ratios underestimate the

metabolic derangement in these patients. Recently, flux through the creatine kinase reaction has been measured in human hearts, and showed that creatine kinase flux is reduced in patients with heart failure [9]. Interestingly, PCr : ATP ratios were also found to be reduced in patients with type 2 diabetes but no evidence of coronary artery disease or impaired cardiac function [10]. Overall, such studies suggest altered energetics as a key mechanism in heart failure.

Over the past decade,  $^{31}\text{P}$ -MRS has been extensively applied in transgenic mouse models targeting cardiac metabolism; it is a particularly useful way in which to study models targeting the creatine/creatine kinase system. Measuring creatine kinase flux in hearts from creatine kinase knockout mice elucidated the relative contributions of the different isoenzymes [11]. Guanidinoacetate-*N*-methyltransferase (GAMT) knockout mice, which lack cardiac creatine, show a mild cardiac phenotype. Using  $^{31}\text{P}$ -MRS, we were able to explain this finding, showing that the precursor of creatine, guanidino-acetate, takes over the role of creatine in these mice [1] (Figure 2). Recently, we found that the presence of supranormal creatine concentrations resulting from overexpression of the cardiac creatine transporter leads to heart failure [12].  $^{31}\text{P}$ -MRS revealed that the free energy change of ATP hydrolysis is reduced in these hearts, explaining contractile dysfunction. Using  $^{31}\text{P}$ -MRS, abnormal cardiac energetics have been characterized in many other transgenic animal models; for example, mice lacking glucose transporter 4 [13] or peroxisome proliferator-activated receptor- $\alpha$  [14]. In mice with a mutation in the myosin heavy chain, the metabolic consequence of the mutation (reduced phosphocreatine) has been detected with  $^{31}\text{P}$ -MRS [15].

In contrast to  $^{31}\text{P}$ -MRS, carbon-13 ( $^{13}\text{C}$ )-MRS can be used to study a much larger number of compounds,

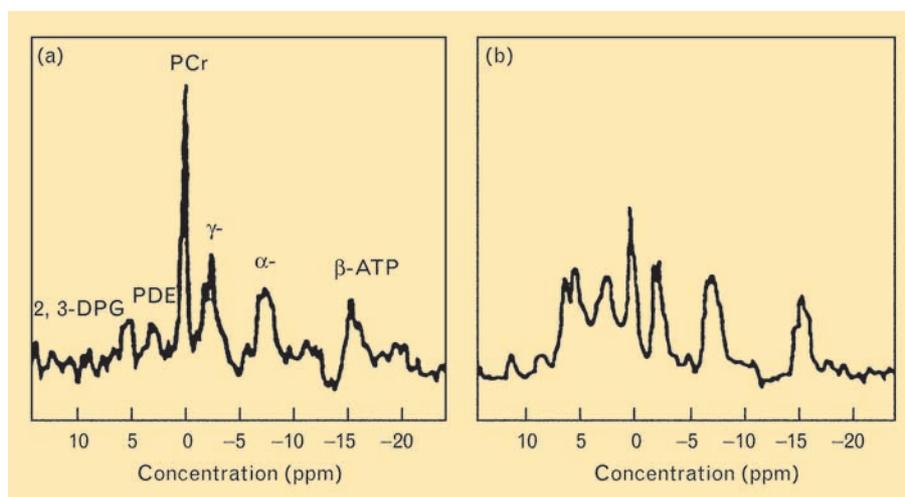


Figure 1. Typical phosphorus-31 magnetic resonance spectra (a) from a volunteer and (b) from a patient with severe dilated cardiomyopathy. In the patient, the phosphocreatine (PCr) : ATP ratio is markedly reduced. 2,3-DPG, 2,3-diphosphoglycerate; PDE, phosphodiester; ppm, parts per million. (Modified from Neubauer et al [6], with permission).

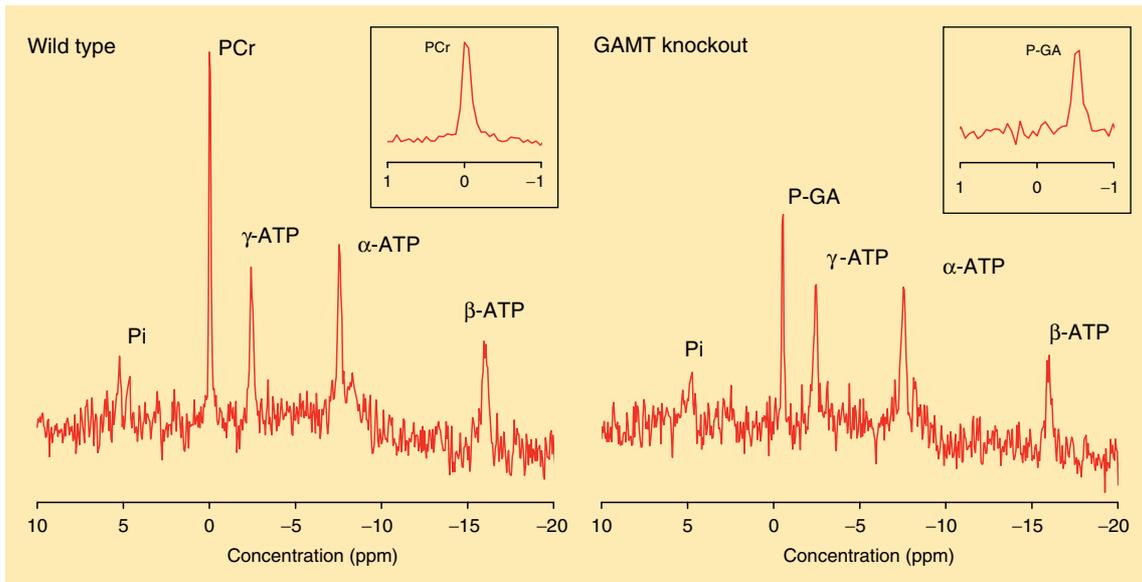


Figure 2. Typical phosphorus-31 magnetic resonance spectra of isolated perfused hearts from (a) a wild-type and (b) a guanidinoacetate-N-methyltransferase (GAMT) knockout mouse. GAMT knockout mice completely lack creatine and therefore no phosphocreatine (PCr) is visible in the spectrum. Instead, the phosphorylated form of guanidino-acetate (P-GA), the precursor of creatine, is visible. The P-GA peak appears just to the right of the position in which PCr appears in normal hearts (see inset).  $\alpha$ -ATP,  $\beta$ -ATP and  $\gamma$ -ATP =  $\alpha$ -,  $\beta$ - and  $\gamma$ -P-atom of ATP; Pi, inorganic phosphate; ppm, parts per million.

including many intermediates of metabolic pathways; it can thus be an extremely powerful tool. However, the sensitivity of this technique is particularly low, because of the low “nuclear magnetic resonance (NMR) visibility” and the low natural abundance of  $^{13}\text{C}$  (approximately 1%). Therefore,  $^{13}\text{C}$ -MRS of the intact heart is usually performed while supplying compounds enriched with  $^{13}\text{C}$ , making it an expensive technique. However, using enriched compounds has

advantages. For example, isolated hearts have been perfused with  $^{13}\text{C}$ -labeled substrates to study tricarboxylic acid cycle kinetics [16]. The use of intravenous infusion of labeled substrates in rats enabled identification of the substrates that the heart uses preferentially [17]. Recently, triacylglycerol storage and turnover rates measured with  $^{13}\text{C}$ -MRS were found to be profoundly different in isolated hearts from diabetic rats compared with those from control

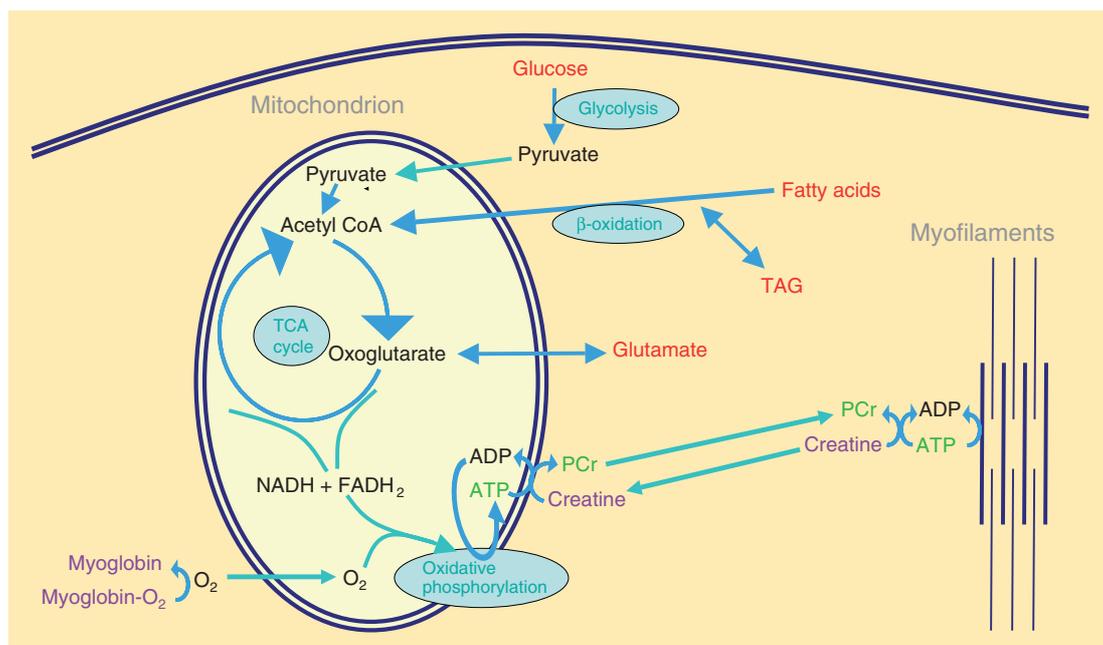


Figure 3. Schematic representation of myocardial metabolism. Metabolites that have been measured with magnetic resonance spectroscopy (MRS) in the intact heart are marked in green (phosphorus-31-MRS), red (carbon-13-MRS) and purple (hydrogen-1/proton]-MRS). CoA, coenzyme A; P-Cr, phosphocreatine; TAG, triacylglycerol; TCA, tricarboxylic acid.

rats [18]. However, because of the low sensitivity associated with  $^{13}\text{C}$ -MRS, in many studies perchloric acid extractions of the heart are used for a more detailed study of cardiac metabolism. With this technique it has been shown that, in myoglobin knockout mice, cardiac substrate metabolism is switched from fatty acid to glucose oxidation [19].

Hydrogen-1 (proton)-MRS has the advantage of relatively high "NMR sensitivity", but  $^1\text{H}$ -MRS of biological tissue is dominated by signal from protons in water. Additional water suppression techniques are therefore required, leading to signal loss. Although  $^1\text{H}$ -MRS can be used to measure many different compounds, its application to study of the metabolism of the intact heart has been limited. Total creatine (phosphocreatine plus free creatine) has been measured in perfused rat hearts [20] and in vivo in humans [21] and, more recently, in the mouse [22]. In patients with heart failure, total creatine concentrations, like phosphocreatine concentrations, were reduced [21].  $^1\text{H}$ -MRS has also been applied to measure oxygenation of myoglobin in isolated mouse hearts [23], and cardiac deoxymyoglobin has been measured in vivo in infarcted swine heart [3]. More recently,  $^1\text{H}$ -MRS examination of perchloric acid extracts from hearts has been used for so called "metabolomics", an approach to measure changes in a large number of metabolites [24].

Figure 3 summarizes myocardial metabolism and identifies the metabolites that have been measured in the intact heart with MRS.

To date, human MRS has been limited to  $^{31}\text{P}$ -MRS and  $^1\text{H}$ -MRS, and has been applied only as a research tool. Better coils and magnets with greater field strength, in addition to application of a localization technique called spectral localization with optimum pointspread function (SLOOP), which allows for better matching of voxels to the curved shape of the heart, should ultimately bring clinical MRS closer to reality. In addition, hyperpolarized  $^{13}\text{C}$ -MRI and/or MRS has been reported [25]. With this technique, the signal-to-noise ratio is enhanced by a factor of  $10^5$  and metabolic  $^{13}\text{C}$  imaging may become a possibility. ■

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