[Frontiers in Bioscience S3, 331-340, January 1, 2011]

MR spectroscopy in heart failure

Cameron Holloway[1], Michiel ten Hove[2], Kieran Clarke[1], Stefan Neubauer[2]

[1]Department of Physiology, Anatomy and Genetics, University of Oxford, United Kingdom, [2]Department of Cardiovascular Medicine, University of Oxford, United Kingdom

TABLE OF CONTENTS

1. ABSTRACT

Magnetic resonance spectroscopy (MRS) is an established technique for the non-invasive assessment of myocardial metabolism. MRS is ideal for the evaluation of heart failure, as it allows quantification of the primary energy source for all myocardial cellular functions (ATP), the energy reserve phosphocreatine (PCr), and the creatine kinase reaction, which maintains cellular energy equilibrium. PCr forms the primary ATP buffer in the cell via the creatine kinase (CK) reaction and is involved in transporting the chemical energy from the ATP-producing mitochondria to the ATP-consuming contractile proteins. Using ^{31}phosphorus (^{31}P) MRS, a low cardiac PCr/ATP has consistently been found in patients with heart failure, supporting the hypothesis that the failing heart is energy starved. The use of ^{1}H MRS has allowed the detection of total creatine, which when combined with ^{31}P MRS, provides an in depth examination of the creatine kinase reaction. MRS signals from ^{31}P, ^{1}H, ^{23}Na and ^{13}C, including novel hyperpolarization techniques, have provided considerable insight into the understanding of energy metabolism in the healthy and diseased heart.

2. INTRODUCTION AND TECHNICAL ASPECTS

Heart failure is the end result of a number of genetic, ischemic and metabolic abnormalities, ultimately leading to complex myocardial alterations, including changes in gene expression, signal transduction, energy metabolism, ion homeostasis and contractile properties. Yet, despite significant technological advances, most routine diagnostic tools for the myocardium are limited to providing anatomical and gross functional information.

Magnetic resonance spectroscopy (MRS) extends cardiac assessment beyond anatomy, function and viability, to include quantitative information on cardiac metabolism. Whilst magnetic resonance imaging (MRI) uses the signal from protons (1H) in water to provide spatial information, MRS can use a number of nuclei to provide biochemical information, making it an attractive tool to study myocardial diseases, including heart failure. These nuclei, which include 1H, 31P, 23Na and 13C, have the property of "nuclear spin" and when placed in a magnetic field and excited at a specific radiofrequency (rf), subsequently give off an rf signal.

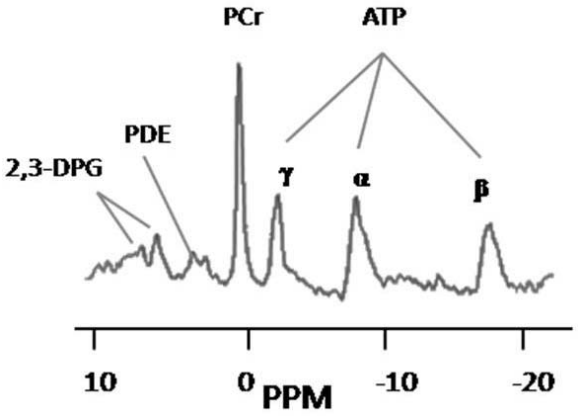

Figure 1. An example of cardiac ^{31}P MR spectrum in a healthy subject showing 2,3 diphosphoglycerate (2,3-DGP), phosphodiester (PDE), phosphocreatine (PCr) and the three phosphorus peaks of ATP (γ, α and β). The spectrum is presented as a function of the precession frequencies divided by the magnetic field strength and expressed on the x-axis in parts per million (ppm).

Nuclei suitable for MRS can provide a signal with a frequency in the range of normal radio waves. The strength of the signal correlates linearly with the strength of the magnetic field. Therefore, MRI and MRS are normally performed in magnets with high field strength. Most clinical MRS studies have been carried out at a magnet strength of 1.5 T (Tesla), although there may be a two-fold better signal-to-noise ratio for spectra at 3T (1). This shows promise of better sensitivity with the emergence of even higher field strength magnets, such as 7 T. In magnets suitable for animal studies, the smaller bore size makes it technically easier to create a strong, homogenous magnetic field. This means magnets used for experimental studies are generally stronger than clinical scanners, typically varying between 4.7 T and 18 T.

The frequency of the signal in MR can be expressed as: $\gamma = \Omega \times B_0$

where γ is the frequency of the signal, B_0 the strength of the magnetic field and Ω a constant specific for the studied nucleus. This property is used in spectroscopy to determine metabolite concentrations and in MR imaging to gain spatial information. By providing a gradient of varying magnetic strengths along the magnet bore, the location of interest can be investigated. Nuclei, even within the same metabolite, resonate at different frequencies depending on their spatial distribution and chemical environment, a phenomenon called chemical shift. For instance, chemical shift allows quantification of the hydrogen nuclei associated with both water and lipids within the same proton spectrum and both phosphocreatine and ATP within the same ^{31}P MR spectrum. A ^{31}P MR spectrum relies on the principle that signal from the three ^{31}P nuclei in ATP have a lower resonance frequency than the signal from the ^{31}P in PCr. Although these differences are small, they allow differentiation of metabolite quantities within the same sample, or voxel, of interest. The spectrum of data is presented as a function of the different precession frequencies divided by the magnetic field strength, and expressed on the x-axis as parts per million (Figure 1). MRS then allows quantification of metabolites by determination of the area under each spectral peak.

3. ^{31}PHOSPHORUS SPECTROSCOPY

The most widely studied nucleus in MRS is ^{31}phosphorus (^{31}P). In a ^{31}P MR spectrum there are over six discernible phosphorus peaks (Figure 1), including those from two important cardiac metabolites, ATP (as three peaks; γ, α and β) and PCr. Phosphodiesters and 2,3-diphosphoglycerate can also be seen. The signal of inorganic phosphate (Pi) is usually hidden in human cardiac ^{31}P MR spectra by the signal from 2,3-diphosphoglycerate from blood in the ventricle, which resonates at a frequency close to Pi, making Pi quantification, as well as determination of the intracellular pH, difficult, if not impossible. The frequency of Pi depends on pH, which can be used in animal work as an indirect measure of intracellular pH. Measuring intracellular pH was first published in the 1970's and is now routinely performed on isolated hearts (2, 3).

There are various methods used for quantification of high energy phosphate molecules detected using spectroscopic techniques. Absolute quantification can be achieved by comparing the signal from the sample with an external reference or by quantifying one of the metabolites, eg. ATP, via a separate method, including high performance liquid chromatography (HPLC). Although PCr can also be measured by HPLC, the true concentrations can be underestimated, as it is highly unstable and degrades rapidly. Absolute quantification of PCr and ATP is possible in isolated heart, however it is more challenging *in vivo*. Because of the difficulties involved when determining absolute quantities using *in vivo* ^{31}P spectroscopy, the ratio of PCr/ATP is more commonly used. Despite this limitation, PCr/ATP is a powerful index of the energetic state of the heart.

4. PROTON SPECTROSCOPY

Using ^{1}H MRS, a wide range of metabolites can be studied. Although ^{1}H MR spectroscopy has been established in the mouse heart *in-vivo*(4), little has been reported for humans in heart failure. ^{1}H MRS allows characterisation of myocardial fat content, lactate, carnitine, myoglobin and total creatine levels, which are reduced in heart failure (5, 6). The combination of ^{1}H and ^{31}P MRS provides a more complete metabolic profile, giving total creatine and high energy phosphate compounds, respectively (7). A major technical challenge in ^{1}H MRS is suppression of the ^{1}H signal from water, given its abundance (55 M) compared with other proton containing molecules that are in millimolar concentrations. Water suppression techniques, however, may cause signal loss from other metabolites.

5. ^{23}SODIUM AND ^{87}RUBIDIUM MR SPECTROSCOPY

^{23}Na MRS can be used to measure intra and extracellular Na^+ and ^{87}Rb MRS calculated to determine

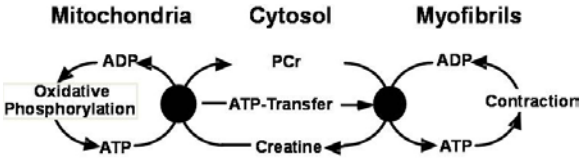

Figure 2. The phosphocreatine/creatine kinase system. ATP synthesized by the mitochondria reacts with creatine (Cr) in a reaction catalyzed by the mitochondrial isoform of creatine kinase (Mito-CK) to form phosphocreatine (PCr) and ADP. PCr diffuses to the myofibrils where the muscle type isoform of creatine kinase (MM-CK) catalyzes the back reaction to form ATP and Cr, maintaining adequately high ATP and low ADP levels to facilitate contraction.

myocardial Na^+/K^+ ATPase activity, Rb^+ being a K^+ congener. Both methods have been used to study ischemic myocardium(8-10), but not to study the failing heart. Experimental *in vivo* cardiac ^{23}Na NMR studies allow determination of the total Na^+ signal, and the intra- and extracellular Na^+ pools can be split into two resonances after the addition of a shift reagent to the perfusate, although ^{23}Na-shift reagents are not yet available for clinical use. ^{23}Na MRI in rats has shown increased sodium in non-viable myocardium, compared with non-scarred, or viable, myocardium, indicating a potential role for this technique in infarct identification without the need for external contrast agents (11). ^{23}Na MRI has also proven useful for identification of solid malignant tumors in human brain that might help curtail the need for invasive biopsies on cardiac tumours if applied to heart (12).

6. HYPERPOLARIZATION

New and innovative hyperpolarization techniques have been developed that allow detection of molecules with low abundance. Even in the strongest magnets, there is relatively low nuclear polarization under usual conditions, hence the low sensitivity of MR. Ways of increasing the polarization of nuclear spins includes increasing the magnetic field strength, decreasing the temperature or specific hyperpolarization techniques, such as dynamic nuclear polarization (DNP). In DNP, radicals and a substance labelled with ^{13}C, are frozen and irradiated close to the electron resonance frequency in a high magnetic field, improving the sensitivity of ^{13}C MR spectroscopy by many orders of magnitude (13, 14). Following dissolution, the resultant “hyperpolarized” solution has an enhanced ^{13}C MR signal that can be used for determination of metabolites and enzyme kinetics in fundamental metabolic pathways of substrate metabolism. To date, this technique has been limited to research applications. Assessment of pyruvate and its metabolites, including lactate, alanine and bicarbonate, have been possible in *in vivo* animal hearts (15-17), with changes in flux through the pyruvate dehydrogenase (PDH) enzyme complex demonstrated following ischaemia-reperfusion. Further work using hyperpolarized pyruvate has demonstrated the ability to assess Krebs cycle metabolism (18) and intracellular pH (19) in real time. Thus, an enormous amount of information can be gained from these techniques. We can speculate that, if this technique were suitable for use in humans, it would have the potential to provide real-time metabolic assessment in heart failure patients and to monitor response to therapies.

7. THE PHOSPHOCREATINE/CREATINE KINASE SYSTEM

Cardiac MRS has been most widely used to study energy metabolism, in particular the phosphocreatine/creatine kinase system. This is highly relevant to the study of heart failure, because available evidence suggests that the failing heart is energy starved (20, 21), and therefore energy metabolism is considered a promising target for new forms of heart failure therapies.

All cellular energy consuming processes are fuelled by the hydrolysis of ATP to ADP:

$$ATP \leftrightarrow ADP + Pi$$

In cardiomyocytes, most of the ATP is synthesised in the mitochondria and reacts with creatine (Cr) in the creatine kinase (CK) reaction to form phosphocreatine (PCr):

$$ATP + Cr \leftrightarrow ADP + PCr + H^+$$

Phosphocreatine (PCr), the other major energy-rich phosphate compound, serves as a reservoir of energy and has at least two additional functions. PCr serves as an energy transport molecule in the creatine kinase/phosphocreatine "energy shuttle" (Figure 2). In mitochondria, a high-energy phosphate bond is transferred from ATP to creatine, producing phosphocreatine and ADP. This reaction is catalyzed by the mitochondrial isoenzyme of creatine kinase. Phosphocreatine, a much smaller molecule than ATP, then diffuses through the cytosol to sites of ATP utilization, including the myofibrils. At the myofibrils, there is a reverse reaction, where ATP used for contraction, forming ADP and creatine. The free creatine then diffuses back to the mitochondria. This "energy shuttle" is essential for cells with high energy consumption, because the low concentration of free cytosolic ADP (40-80 μM) does not provide the necessary back-diffusion to mitochondria, whereas free creatine concentrations are at least two orders of magnitude higher. The second crucial cellular function of phosphocreatine and the creatine kinase reaction is to keep the free cytosolic ADP concentration low. The concentration of free ADP cannot be determined directly, but is calculated on the creatine kinase equilibrium equation:

$$ADP = ([ATP]) \times [creatine]) / ([phosphocreatine]) \times [H^+] \times K_{eq})$$

Where $[H^+]$ is the intracellular proton concentration and K_{eq} is the equilibrium constant of the creatine kinase reaction.

A low free cytosolic ADP concentration is essential for the maintenance of normal contractile function, as ADP directly determines the free energy

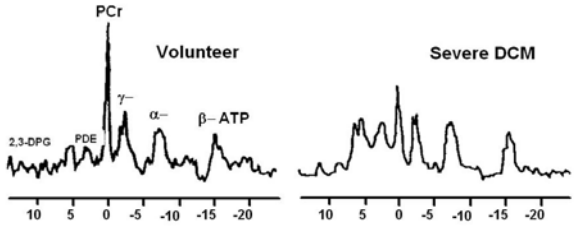

Figure 3. ^{31}P Magnetic Resonance Spectroscopy from a normal volunteer, with a normal PCr/ATP ratio (left) and a patient with dilated cardiomyopathy (DCM) and severely reduced PCr /ATP (right). Reproduced from EHJ 16:115-118, 1995 with permission.

change of ATP hydrolysis (ΔG, kJ/mol), which is a measure of the energy that is released on ATP hydrolysis:

$\Delta G_{ATP} = \Delta G_0 + RT \ln$ (ADP x inorganic phosphate) / ATP
ΔG_0 is the standard free energy at 37°C, $[Mg^{2+}] = 1$ mM, R is the universal gas constant and T is the temperature [K].

Normally ΔG is in the order of -58 kJ/mol. Various intracellular enzymes, such as the SR-Ca^{2+}-ATPase, stop functioning if ΔG falls below a threshold of about -52 kJ / mol.

8. CARDIAC MRS IN THE HEALTHY HEART

The first cardiac ^{31}P NMR spectra of the heart were published in the 1970s using isolated perfused rat hearts (22) and the first publication on *in vivo* cardiac spectroscopy was in 1980 on rat heart (23). The technique is now well established in specialized research centres for the non-invasive assessment of human cardiac high energy phosphate metabolism. By using an external standard, absolute concentrations can be determined, however ratios of PCr/ATP are most commonly reported. Experimental determination of PCr/ATP has yielded normal values of approximately 2, however *in vivo* human MRS studies have reported a wide range of normal values, from 1.1 to 2.5, with evidence of a decrease in high-energy phosphate metabolites with increasing age (24-26). The wide variation in normal values reflects the differences in acquisition, post-processing techniques and correction factors, such that institutions need to establish their own normal ranges. Although direct comparison between sites is a problem, it will be essential if cardiac MRS is to become clinically useful in the long term.

Absolute concentrations of myocardial PCr and ATP are roughly similar in different species, being ~10 μmol/g wet weight for PCr and ~6 μmol/g wet weight for ATP in both canine and human heart (7, 27-30). In one *in vivo* canine study, ^{1}H and ^{31}P MRS were used to measure myocardial PCr, ATP and Cr and to calculate the free ADP concentration, which was 0.11 μmol/g wet weight with ΔG_{ATP} -56.3 kJ/mol. Total Cr concentrations in human myocardium have been measured using ^{1}H MRS to be ~ 27 μmol/g wet weight (31, 32). Like the PCr and ATP concentrations, the forward reaction velocity of the creatine kinase reaction, k_{for}, appears to be similar in healthy rat, dog and porcine myocardium at ~ 0.5 s^{-1}. In human myocardium, a k_{for} of ~0.3 s^{-1} and a CK flux of 3.2 μmol/g wet weight per second have been reported, with flux not altered by an increased rate pressure product during dobutamine infusion (33).

9. CARDIAC MRS IN THE FAILING HEART

Cardiac MRS has been widely used for the assessment of myocardial metabolism in systolic dysfunction. Figure 3 shows examples of ^{31}P-MRS spectra from a healthy subject and from a heart failure patient. On considering all heart failure MRS studies, clinical and experimental, it becomes clear that, regardless of the aetiology of heart failure, the PCr/CK system is impaired. Myocardial PCr, Cr_{total} and CK enzyme levels are consistently low at all stages of heart failure and can precede cardiac dysfunction.(34) Indeed, abnormalities in cardiac energy metabolism may underlie the pathophysiology of heart failure with preserved systolic function; "diastolic heart failure" (35, 36). Supporting this theory, cardiac PCr/ATP was reduced in 37 patients with heart failure and preserved systolic function, versus control subjects (1.57 ± 0.52 vs. 2.14 ± 0.63), indicating reduced energy reserves (37).

Flux through the CK reaction also decreases in failing myocardium. However, reduced CK kinetics is only found in more advanced stages of heart failure. The PCr/CK system appears to be designed to keep the ATP levels constant for as long as possible, such that reduced ATP levels are only found in severe, end-stage heart failure.

9.1. Heart failure due to ischemic heart disease

The role of MRS for assessment of heart failure caused by ischemic heart disease has been limited by low spatial resolution (38). Due to the difficulties in assessing inhomogeneous diseases, such as ischemic heart disease (IHD), there have been a limited number of studies examining the myocardium of patients with heart failure due to IHD (39). ^{1}H and ^{31}P MRS have been used to identify ischemic myocardium and established infarction (38, 40, 41). Handgrip exercise testing in 16 patients with left anterior descending artery ischemia showed reduced PCr/ATP ratios in ischemic myocardium, and the energy deficit reversed after revascularization (42). A study by *Hardy et.al.* found significantly reduced PCr/ATP in patients with dilated cardiomyopathies secondary to ischemic heart disease. A recent study in 15 patients with previous anterior myocardial infarction, demonstrated that mean myocardial ATP and PCr concentrations were 39% to 44% lower in the infarct territory, measured using ^{31}P-MRS, compared with control subjects (PCr 5.4 ± 1.2 versus 9.6 ± 1.1 mol/g wet weight).(43) In these subjects, the myocardial CK rate constant, k, was normal in myocardial infarct patients compared with control subjects, as was PCr/ATP (1.74 ± 0.27 in the infarct group, versus 1.87 ± 0.45 in the control group). The infarct group also had a 50% lower CK flux with the conclusion that ATP supply was significantly reduced as a result of substrate depletion, probably as a result of myocyte loss. Within the same heart,

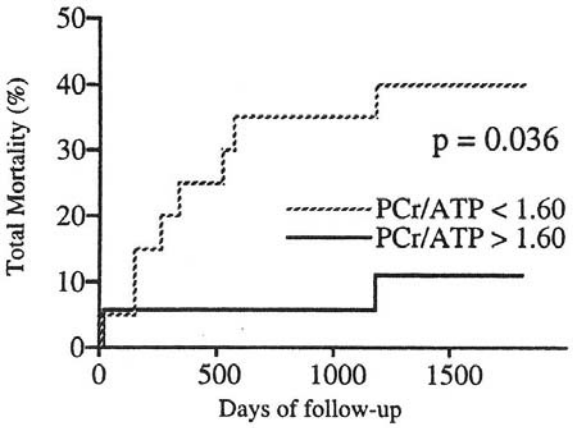

Figure 4. Kaplan-Meier life table analysis for total mortality of patients with dilated cardiomyopathy. Patients divided into two groups according to PCr to ATP ratios (<1.60 and >1.60). Patients with a reduced PCr to ATP ratio had an increased mortality over the study period compared to those with a higher ratio (p=0.036). Reproduced from Circulation 1997; 96; 2190-6 with permission from the American Heart Association.

patients with IHD can have full thickness infarction, subendocardial infarcts, hibernating and normal myocardium, but the voxel sizes used in current MRS techniques are too large to consistently distinguish these compartments.

Experimental MRS studies in heart failure due to ischemic heart disease have been performed. In one study, rats were subjected to chronic myocardial infarction (MI) induced by ligation of the left anterior descending coronary artery (LAD). After eight weeks, hearts were excised, isolated and perfused in the Langendorff mode. Using ^{31}P MRS, PCr and ATP levels, as well as the CK reaction velocity, in residual intact myocardium were measured. Although ATP levels were unchanged, PCr levels were significantly reduced by 31% and flux through the CK reaction was decreased by 50%. Interestingly, calculated [ADP] was unchanged. Another study used ^{31}P-MRS to investigate the effect of mesenchymal stem cells to repair myocardial infarction in rat heart, to show that stem cell therapy spared myocardial stores of PCr (44).

MRS has fundamental advantages over current diagnostic methods for assessment of viability, including nuclear perfusion scanning, dobutamine-stress echocardiography and positron emission tomography: it is radiation free, requires no intravenous substances, no physical or pharmacological stress and uses intrinsic contrast to distinguish between viable and nonviable myocardium. Therefore, if there were a significant improvement in the temporal and spatial resolution of the technique (leading to smaller voxel sizes), ^{31}P-MRS theoretically could have widespread clinical application in IHD for the assessment of myocardial viability. Further studies are required to determine if changes in PCr, ATP or ATP turnover rates could be used as markers for diagnosis or prognosis in IHD.

9.2. Hypertrophic cardiomyopathy

Cardiac MRS has been used to evaluate patients with hypertrophic cardiomyopathy and their asymptomatic relatives, showing reduced PCr/ATP in both groups (45, 46, 47). This suggests that abnormalities in myocardial energy metabolism precede morphological abnormalities and also suggests a possible role for ^{31}P MRS in screening. These findings have led to the recent paradigm that hypertrophic cardiomyopathy is a disease of compromised energetics (48), a paradigm further supported by experiments on mice with a mutation at position 403 in the β-cardiac myosin heavy chain, which leads to familiar hypertrophic cardiomyopathy (49). Such mice show impaired diastolic function. ^{31}P MRS examination revealed that myocardial PCr/ATP ratios were reduced, similar to the reduction found in HCM patients. The reduction in PCr/ATP was caused by reduced PCr levels, whilst ATP levels were essentially unaltered. These mice also showed increased Pi levels and the calculated ΔG_{ATP} was reduced, suggesting that energetic abnormalities may contribute to the diastolic dysfunction observed. Similar results were obtained by the same group on mice bearing another mutation associated with a clinical form of familial hypertrophic cardiomyopathy, the R92Q mutation in cardiac troponin T (50).

Differentiating between pathological left ventricular hypertrophy caused by hypertrophic cardiomyopathy or hypertensive heart disease on the one hand, and adaptive hypertrophy in athletes on the other, can be clinically challenging. MRS may develop to be a useful tool for this clinical challenge, as studies using ^{31}P MRS have shown normal high energy phosphate ratios in athletes, but impaired PCr/ATP in hypertensive patients and those with left ventricular pressure overload (51-55).

9.3. Dilated cardiomyopathy

MRS has been extensively evaluated in patients with dilated cardiomyopathy, with PCr/ATP correlating with the clinical severity of heart failure and having greater prognostic value than left ventricular ejection fraction (41, 56). Reduced PCr/ATP not only predicts symptoms in patients with dilated cardiomyopathy, but also predicts mortality (Figure 4, 56). In one study, absolute concentrations of PCr and ATP were measured in DCM patients (29). The reduction in PCr/ATP found in other studies was confirmed but, importantly, it was also shown that both PCr and ATP were decreased by 51% and 35%, respectively. This implies that the reduction in PCr/ATP observed in these patients underestimated the true biochemical changes in this disease. A recent study investigating the effects of exercise training on myocardial function and energetics in dilated cardiomyopathy found improvement in left ventricular end-systolic volume and ejection fraction, however no change in cardiac PCr/ATP levels (53). The authors concluded that the absence of deterioration in myocardial energetics suggested that the exercise training was not energy-costly to the myocardium, supporting exercise therapy in dilated cardiomyopathy.

Using ^{1}H MRS it has been shown that total myocardial creatine content in patients with DCM is also

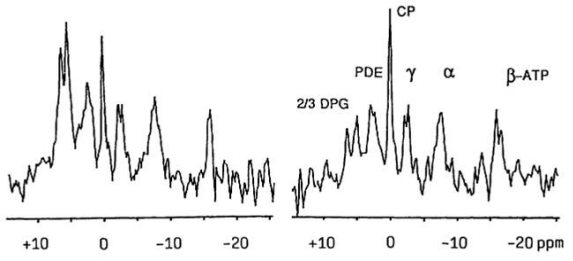

Figure 5. ^{31}P spectra of a patient with dilated cardiomyopathy before (left panel) and 18 weeks after treatment with digitalis, diuretics, ACE inhibitors and β-blockers (right panel). The clinical status improved from New York Heart Association class III to II, while PCr/ATP ratio increased. Reproduced from Circulation 1992; 86: 1810-1818 with permission from the American Heart Association.

reduced, from 28 μmol/g wet weight in control subjects to 16 μmol/g wet weight in patients (32), possibly to maintain ΔG_{ATP} at normal levels.

9.4. Diabetes

In support of a metabolic basis for diabetic cardiomyopathy, patients with diabetes and normal systolic function have low cardiac PCr/ATP ratios, unrelated to the duration of diabetes and independent of microvascular dysfunction (36, 57-59). Studies using ^{1}H MRS have shown reduced myocardial triglycerides after a low calorie diet in diabetic patients, and similar studies in heart failure patients are awaited (60-62). ^{1}H MRS has the potential to help define the effect of triglyceride metabolism, modified by diet and drugs, on the failing heart.

9.5. Heart transplantation

A study using cardiac ^{31}P-MRS has shown that functional recovery after human heart transplantation is related to the metabolic condition of the donor heart (63). Animal studies have suggested that ^{31}P-MRS could be suitable for the detection of heart rejection after transplantation (64-66). Therefore, it was hoped that the MRS could provide a non-invasive diagnosis of the extent of graft rejection, and thus eliminate the need for repetitive endomyocardial biopsies. Clinical studies could not confirm this, unfortunately. Although the PCr/ATP ratios were reduced in transplanted hearts with histological signs of rejection, there was no correlation with the histological severity of rejection (65, 67). A further study using exercise phosphorus MRS studies in heart transplant patients showed a decrease in PCr/ATP during exercise, although this was only in some patients with no correlation made with histological rejection (68).

10. MONITORING HEART FAILURE THERAPIES

Medical treatment for heart failure has included neuro-hormonal antagonism, with associated improvement in outcome after ACE inhibitors, angiotensin II receptor blockers and β-blockers. A small group of patients with dilated cardiomyopathy was studied before and after initiation of heart failure therapy, with PCr to ATP ratios improving from 1.51 ± 0.32 to 2.15 ± 0.27 within 3 months, in line with an improvement in clinical status (Figure 5, 41). This demonstrates that such heart failure therapies can ameliorate the metabolic deficit in heart failure, partially through energy-sparing effects, and also shows that cardiac spectroscopy can potentially be used to guide heart failure treatment. In a group of heart failure patients with ischemic and non-ischaemic aetiologies, a study using a substrate modulator, Trimetazidine, which switches energy metabolism from fatty acid to glucose oxidation, found a 33% improvement in PCr/ATP ratios from 1.35 ± 0.33 to 1.80 ± 0.50 (69). Although small, this randomised, double-blind, cross-over study in twelve heart failure patients, already on standard heart failure therapy, suggests promising results for this drug, and other potential therapies, that modulate myocardial substrate metabolism.

11. TECHNICAL DEVELOPMENTS

Technical developments have improved spectral signal. Skeletal muscle from the chest wall can contaminate the cardiac spectra and, therefore, localization techniques are required, that can result in further signal loss. Improved spectral quality can be obtained with saturation bands placed over the skeletal muscle and liver during acquisition to reduce contamination (70). Furthermore, SLOOP (spectral localization with optimum point spread function) is a localization method employed to reduce contamination from adjacent tissue, including skeletal muscle and liver, but is difficult to implement (28). Matching voxel shape to the curvature of the heart helps to increase the signal-to-noise and avoids contamination from myocardial tissue outside the region of interest, from skeletal muscle and from blood. Nuclear Overhauser Enhancement (NOE) can additionally be applied to improve signal-to-noise (71). The evolution of higher field strength magnets may allow smaller voxel sizes with improved signal-to-noise and reduced adjacent tissue contamination (70).

12. LIMITATIONS CURRENTLY PREVENTING WIDESPREAD CLINICAL APPLICATIONS

Technical limitations have so far prevented the widespread clinical application of MRS, in particular the limited temporal and spatial resolution, which result in high variability and low reproducibility, especially at low field strengths. The main reason for this is the intrinsically low signal of MRS compared with MRI, owing to the lower concentrations and MR sensitivity of the nuclei being studied. Concentrations of metabolites that are studied in MRS are generally several orders of magnitude lower than those of proton in water (Table 1). The lower nuclei concentrations and signal result in low signal-to-noise ratios. The limitations mean that larger voxel sizes and long scan times are needed to produce sufficient signal-to-noise ratios. This has important practical considerations when assessing heart failure patients, as large voxel sizes are only suitable for studying predominantly homogenous myocardial diseases. Additionally, long scan times are often not tolerated by heart failure patients, who have to lie prone in the magnet.

Table 1. Nuclei for MRS of the heart

Nucleus	Natural abundance	Relative MR sensitivity	Myocardial tissue concentrations	Relative signal intensity compared with H_2O
^{1}H	99.98 %	1	H_2O 55 M, Cr up to 90 mM	Cr; 1:1200
^{31}P	100 %	0.066	Up to 20 mM (PCr)	1:80000
^{13}C	1.1 %	0.016	Labelled compounds, several mM	1:100000000
^{23}Na	100 %	0.093	~11 mM (intracellular); 140 mM (extracellular)	1:100000

13. CONCLUSION

Cardiac ^{31}P and ^{1}H spectroscopy have improved our understanding of high energy phosphate and creatine metabolism in heart failure, but the technique remains a research tool. The development of new spectroscopy sequences, higher field strengths and better coil design may enable high-resolution metabolic imaging as a routine diagnostic tool in cardiology for patients with heart failure. This could include examination of high energy phosphate metabolism, the use of ^{1}H MRS to study the CK reaction velocity and myocardial triglyceride content (especially in obese and diabetic patients) and hyperpolarization techniques to determine substrate metabolism. The advent of higher field strength magnets and improved sensitivity may allow wider use of MRS in the assessment of myocardial disorders and may deliver the promise of a tool suitable for clinical use. In the meantime, ^{31}P MRS will continue to be used as a powerful research tool for assessment of cardiac energy metabolism *in vivo*.

14. ACKNOWLEDGMENT

There are no conflicts of interest to declare

15. REFERENCES

1. D. J. Tyler, L. E. Hudsmith, K. Clarke, S. Neubauer and M. D. Robson: A comparison of cardiac (31)P MRS at 1.5 and 3 T. *NMR Biomed*, 21(8), 793-8 (2008)

2. P. B. Garlick, G. K. Radda, P. J. Seeley and B. Chance: Phosphorus NMR studies on perfused heart. *Biochem Biophys Res Commun*, 74(3), 1256-62 (1977)

3. W. E. Jacobus, G. J. t. Taylor, D. P. Hollis and R. L. Nunnally: Phosphorus nuclear magnetic resonance of perfused working rat hearts. *Nature*, 265(5596), 756-8 (1977)

4. J. E. Schneider, D. J. Tyler, M. ten Hove, A. E. Sang, P. J. Cassidy, A. Fischer, J. Wallis, L. M. Sebag-Montefiore, H. Watkins, D. Isbrandt, K. Clarke and S. Neubauer: *In vivo* cardiac 1H-MRS in the mouse. *Magn Reson Med*, 52(5), 1029-35 (2004)

5. M. Kankaanpaa, H. R. Lehto, J. P. Parkka, M. Komu, A. Viljanen, E. Ferrannini, J. Knuuti, P. Nuutila, R. Parkkola and P. Iozzo: Myocardial triglyceride content and epicardial fat mass in human obesity: relationship to left ventricular function and serum free fatty acid levels. *J Clin Endocrinol Metab*, 91(11), 4689-95 (2006)

6. J. S. Ingwall, M. F. Kramer, M. A. Fifer, B. H. Lorell, R. Shemin, W. Grossman and P. D. Allen: The creatine kinase system in normal and diseased human myocardium. *N Engl J Med*, 313(17), 1050-4 (1985)

7. P. A. Bottomley, E. Atalar and R. G. Weiss: Human cardiac high-energy phosphate metabolite concentrations by 1D-resolved NMR spectroscopy. *Magn Reson Med*, 35(5), 664-70 (1996)

8. H. R. Cross, G. K. Radda and K. Clarke: The role of Na+/K+ ATPase activity during low flow ischemia in preventing myocardial injury: a 31P, 23Na and 87Rb NMR spectroscopic study. *Magn Reson Med*, 34(5), 673-85 (1995)

9. M. Ten Hove and C. J. A. Van Echteld: Limited effects of post-ischemic NHE blockade on $[Na^+]_i$ and pH_i in rat hearts explain its lack of cardioprotection. *Cardiovasc Res*, 61(3), 522-529 (2004)

10. V. V. Kupriyanov and M. L. Gruwel: Rubidium-87 magnetic resonance spectroscopy and imaging for analysis of mammalian K+ transport. *NMR Biomed*, 18(2), 111-24 (2005)

11. M. Horn, C. Weidensteiner, H. Scheffer, M. Meininger, M. de Groot, H. Remkes, C. Dienesch, K. Przyklenk, M. von Kienlin and S. Neubauer: Detection of myocardial viability based on measurement of sodium content: A (23)Na-NMR study. *Magn Reson Med*, 45(5), 756-64 (2001)

12. R. Ouwerkerk, K. B. Bleich, J. S. Gillen, M. G. Pomper and P. A. Bottomley: Tissue sodium concentration in human brain tumors as measured with 23Na MR imaging. *Radiology*, 227(2), 529-37 (2003) doi

13. J. H. Ardenkjaer-Larsen, B. Fridlund, A. Gram, G. Hansson, L. Hansson, M. H. Lerche, R. Servin, M. Thaning and K. Golman: Increase in signal-to-noise ratio of > 10,000 times in liquid-state NMR. *Proc Natl Acad Sci U S A*, 100(18), 10158-63 (2003)

14. D. Tyler, M. Schroeder, L. Cochlin, K. Clarke and G. K. Radda: Application of Hyperpolarized Magnetic Resonance in the Study of Cardiac Metabolism *Applied Magnetic Resonance*, 34, 523-531 (2008)

15. K. Golman, J. S. Petersson, P. Magnusson, E. Johansson, P. Akeson, C. M. Chai, G. Hansson and S. Mansson: Cardiac metabolism measured noninvasively by hyperpolarized 13C MRI. *Magn Reson Med*, 59(5), 1005-13 (2008)

16. M. A. Schroeder, L. E. Cochlin, L. C. Heather, K. Clarke, G. K. Radda and D. J. Tyler: *In vivo* assessment of

pyruvate dehydrogenase flux in the heart using hyperpolarized carbon-13 magnetic resonance. *Proc Natl Acad Sci U S A*, 105(33), 12051-6 (2008)

17. D. J. Tyler, M. A. Schroeder, L. E. Cochlin, K. Clarke and G. K. Radda: Application of hyperpolarized magnetic resonance in the study of cardiac metabolism. *Appl. Magn. Reson.*(34), 523-531 (2008)

18. M. A. Schroeder, H. J. Atherton, D. R. Ball, M. A. Cole, L. C. Heather, J. L. Griffin, K. Clarke, G. K. Radda and D. J. Tyler: Real-time assessment of Krebs cycle metabolism using hyperpolarized 13C magnetic resonance spectroscopy. *FASEB J*, 23(8), 2529-38 (2009)

19. M. A. Schroeder, P. Swietach, H. J. Atherton, F. A. Gallagher, P. Lee, G. K. Radda, K. Clarke and D. J. Tyler: Measuring intracellular pH in the heart using hyperpolarized carbon dioxide and bicarbonate: a 13C and 31P magnetic resonance spectroscopy study. *Cardiovasc Res*, 86(1), 82-91

20. J. S. Ingwall and R. G. Weiss: Is the failing heart energy starved? On using chemical energy to support cardiac function. *Circ Res*, 95(2), 135-45 (2004)

21. S. Neubauer: The failing heart--an engine out of fuel. *N Engl J Med*, 356(11), 1140-51 (2007)

22. P. B. Garlick, G. K. Radda and P. J. Seeley: Phosphorus NMR studies on perfused heart. *Biochem Biophys Res Commun*, 74(3), 1256-62 (1977)

23. T. H. Grove, J. J. Ackerman, G. K. Radda and P. J. Bore: Analysis of rat heart *in vivo* by phosphorus nuclear magnetic resonance. *Proc Natl Acad Sci U S A*, 77(1), 299-302 (1980)

24. H. Kostler, W. Landschutz, S. Koeppe, T. Seyfarth, C. Lipke, J. Sandstede, M. Spindler, M. von Kienlin, D. Hahn and M. Beer: Age and gender dependence of human cardiac phosphorus metabolites determined by SLOOP 31P MR spectroscopy. *Magn Reson Med*, 56(4), 907-11 (2006)

25. P. A. Bottomley: MR spectroscopy of the human heart: the status and the challenges. *Radiology*, 191(3), 593-612 (1994)

26. M. Okada, K. Mitsunami, T. Inubushi and M. Kinoshita: Influence of aging or left ventricular hypertrophy on the human heart: contents of phosphorus metabolites measured by 31P MRS. *Magn Reson Med*, 39(5), 772-82 (1998)

27. P. A. Bottomley and R. G. Weiss: Noninvasive localized MR quantification of creatine kinase metabolites in normal and infarcted canine myocardium. *Radiology*, 219(2), 411-8 (2001)

28. M. Meininger, W. Landschutz, M. Beer, T. Seyfarth, M. Horn, T. Pabst, A. Haase, D. Hahn, S. Neubauer and M. von Kienlin: Concentrations of human cardiac phosphorus metabolites determined by SLOOP 31P NMR spectroscopy. *Magn Reson Med*, 41(4), 657-63 (1999)

29. M. Beer, T. Seyfarth, J. Sandstede, W. Landschutz, C. Lipke, H. Kostler, M. von Kienlin, K. Harre, D. Hahn and S. Neubauer: Absolute concentrations of high-energy phosphate metabolites in normal, hypertrophied, and failing human myocardium measured noninvasively with ^{31}P-SLOOP magnetic resonance spectroscopy. *J Am Coll Cardiol*, 40(7), 1267-74 (2002)

30. I. Nakae, K. Mitsunami, T. Yabe, T. Inubushi, S. Morikawa, S. Matsuo, T. Koh and M. Horie: Detection of metabolic abnormality in asynergic regions of ischemic human myocardium using ^{31}P and ^{1}H magnetic resonance spectroscopy. *J Cardiovasc Magn Reson*, 6(3), 685-96 (2004)

31. P. A. Bottomley and R. G. Weiss: Non-invasive magnetic-resonance detection of creatine depletion in non-viable infarcted myocardium. *Lancet*, 351(9104), 714-8 (1998)

32. I. Nakae, K. Mitsunami, T. Omura, T. Yabe, T. Tsutamoto, S. Matsuo, M. Takahashi, S. Morikawa, T. Inubushi, Y. Nakamura, M. Kinoshita and M. Horie: Proton magnetic resonance spectroscopy can detect creatine depletion associated with the progression of heart failure in cardiomyopathy. *J Am Coll Cardiol*, 42(9), 1587-93 (2003)

33. R. G. Weiss, G. Gerstenblith and P. A. Bottomley: ATP flux through creatine kinase in the normal, stressed, and failing human heart. *Proc Natl Acad Sci U S A*, 102(3), 808-13 (2005)

34. M. Spindler, K. W. Saupe, M. E. Christe, H. L. Sweeney, C. E. Seidman, J. G. Seidman and J. S. Ingwall: Diastolic dysfunction and altered energetics in the alphaMHC403/+ mouse model of familial hypertrophic cardiomyopathy. *J Clin Invest*, 101(8), 1775-83 (1998)

35. R. Tian: Energetic basis of diastolic dysfunction. *MAGMA*, 6(2-3), 129-31 (1998)

36. M. Scheuermann-Freestone, P. L. Madsen, D. Manners, A. M. Blamire, R. E. Buckingham, P. Styles, G. K. Radda, S. Neubauer and K. Clarke: Abnormal cardiac and skeletal muscle energy metabolism in patients with type 2 diabetes. *Circulation*, 107(24), 3040-6 (2003)

37. T. T. Phan, K. Abozguia, G. Nallur Shivu, G. Mahadevan, I. Ahmed, L. Williams, G. Dwivedi, K. Patel, P. Steendijk, H. Ashrafian, A. Henning and M. Frenneaux: Heart failure with preserved ejection fraction is characterized by dynamic impairment of active relaxation and contraction of the left ventricle on exercise and associated with myocardial energy deficiency. *J Am Coll Cardiol*, 54(5), 402-9 (2009)

38. M. Beer, M. Viehrig, T. Seyfarth, J. Sandstede, C. Lipke, T. Pabst, W. Kenn, K. Harre, M. Horn, W. Landschutz, M. von Kienlin, S. Neubauer and D. Hahn:

[Cardiac energy metabolism in heart valve diseases with 31P MR spectroscopy]. *Radiologe*, 40(2), 162-7 (2000)

39. C. J. Hardy, R. G. Weiss, P. A. Bottomley and G. Gerstenblith: Altered myocardial high-energy phosphate metabolites in patients with dilated cardiomyopathy. *Am Heart J*, 122(3 Pt 1), 795-801 (1991)

40. F. Sardanelli, F. Zandrino, G. Molinari, S. Cordone, L. Delfino and F. Levrero: Magnetic resonance spectroscopy of ischemic heart disease. *Rays*, 24(1), 149-64 (1999)

41. S. Neubauer, T. Krahe, R. Schindler, M. Horn, H. Hillenbrand, C. Entzeroth, H. Mader, E. P. Kromer, G. A. Riegger, K. Lackner and *et al.*: 31P magnetic resonance spectroscopy in dilated cardiomyopathy and coronary artery disease. Altered cardiac high-energy phosphate metabolism in heart failure. *Circulation*, 86(6), 1810-8 (1992)

42. R. G. Weiss, P. A. Bottomley, C. J. Hardy and G. Gerstenblith: Regional myocardial metabolism of high-energy phosphates during isometric exercise in patients with coronary artery disease. N Engl J Med, 323(23), 1593-600 (1990)

43. P. A. Bottomley, K. C. Wu, G. Gerstenblith, S. P. Schulman, A. Steinberg and R. G. Weiss: Reduced myocardial creatine kinase flux in human myocardial infarction: an in vivo phosphorus magnetic resonance spectroscopy study. Circulation, 119(14), 1918-24 (2009)

44. M. Gnecchi, H. He, L. G. Melo, N. Noiseaux, F. Morello, R. A. de Boer, L. Zhang, R. E. Pratt, V. J. Dzau and J. S. Ingwall: Early beneficial effects of bone marrow-derived mesenchymal stem cells overexpressing Akt on cardiac metabolism after myocardial infarction. Stem Cells, 27(4), 971-9 (2009)

45. J. G. Crilley, E. A. Boehm, E. Blair, B. Rajagopalan, A. M. Blamire, P. Styles, W. J. McKenna, I. Ostman-Smith, K. Clarke and H. Watkins: Hypertrophic cardiomyopathy due to sarcomeric gene mutations is characterized by impaired energy metabolism irrespective of the degree of hypertrophy. *J Am Coll Cardiol*, 41(10), 1776-82 (2003)

46. W. I. Jung, T. Hoess, M. Bunse, S. Widmaier, L. Sieverding, J. Breuer, J. Apitz, O. Schmidt, F. van Erckelens, G. J. Dietze and O. Lutz: Differences in cardiac energetics between patients with familial and nonfamilial hypertrophic cardiomyopathy. *Circulation*, 101(12), E121 (2000)

47. G. N. Shivu, K. Abozguia, T. T. Phan, I. Ahmed, A. Henning and M. Frenneaux: (31)P magnetic resonance spectroscopy to measure *in vivo* cardiac energetics in normal myocardium and hypertrophic cardiomyopathy: Experiences at 3T. *Eur J Radiol* (2008)

48. H. Ashrafian, C. Redwood, E. Blair and H. Watkins: Hypertrophic cardiomyopathy:a paradigm for myocardial energy depletion. *Trends Genet*, 19(5), 263-8 (2003)

49. M. Spindler, K. W. Saupe, M. E. Christe, H. L. Sweeney, C. E. Seidman, J. G. Seidman and J. S. Ingwall: Diastolic dysfunction and altered energetics in the alphaMHC403/+ mouse model of familial hypertrophic cardiomyopathy. *J Clin Invest*, 101(8), 1775 - 1783 (1998)

50. M. M. Javadpour, J. C. Tardiff, I. Pinz and J. S. Ingwall: Decreased energetics in murine hearts bearing the R92Q mutation in cardiac troponin T. *J Clin Invest*, 112(5), 768-75 (2003)

51. H. J. Lamb, H. P. Beyerbacht, A. van der Laarse, B. C. Stoel, J. Doornbos, E. E. van der Wall and A. de Roos: Diastolic dysfunction in hypertensive heart disease is associated with altered myocardial metabolism. *Circulation*, 99(17), 2261-7 (1999)

52. G. Perseghin, F. De Cobelli, A. Esposito, G. Lattuada, I. Terruzzi, A. La Torre, E. Belloni, T. Canu, P. Scifo, A. Del Maschio, L. Luzi and G. Alberti: Effect of the sporting discipline on the right and left ventricular morphology and function of elite male track runners: a magnetic resonance imaging and phosphorus 31 spectroscopy study. *Am Heart J*, 154(5), 937-42 (2007)

53. M. Beer, D. Wagner, J. Myers, J. Sandstede, H. Kostler, D. Hahn, S. Neubauer and P. Dubach: Effects of exercise training on myocardial energy metabolism and ventricular function assessed by quantitative phosphorus-31 magnetic resonance spectroscopy and magnetic resonance imaging in dilated cardiomyopathy. *J Am Coll Cardiol*, 51(19), 1883-91 (2008)

54. B. M. Pluim, H. J. Lamb, H. W. Kayser, F. Leujes, H. P. Beyerbacht, A. H. Zwinderman, A. van der Laarse, H. W. Vliegen, A. de Roos and E. E. van der Wall: Functional and metabolic evaluation of the athlete's heart by magnetic resonance imaging and dobutamine stress magnetic resonance spectroscopy. *Circulation*, 97(7), 666-72 (1998)

55. A. Gupta, V. P. Chacko and R. G. Weiss: Abnormal energetics and ATP depletion in pressure-overload mouse hearts: *in vivo* high-energy phosphate concentration measures by noninvasive magnetic resonance. *Am J Physiol Heart Circ Physiol*, 297(1), H59-64 (2009)

56. S. Neubauer, M. Horn, M. Cramer, K. Harre, J. B. Newell, W. Peters, T. Pabst, G. Ertl, D. Hahn, J. S. Ingwall and K. Kochsiek: Myocardial phosphocreatine-to-ATP ratio is a predictor of mortality in patients with dilated cardiomyopathy. *Circulation*, 96(7), 2190-6 (1997)

57. M. Diamant, H. J. Lamb, Y. Groeneveld, E. L. Endert, J. W. Smit, J. J. Bax, J. A. Romijn, A. de Roos and J. K. Radder: Diastolic dysfunction is associated with altered myocardial metabolism in asymptomatic normotensive patients with well-controlled type 2 diabetes mellitus. *J Am Coll Cardiol*, 42(2), 328-35 (2003)

58. B. Metzler, M. F. Schocke, P. Steinboeck, C. Wolf, W. Judmaier, M. Lechleitner, P. Lukas and O. Pachinger: Decreased high-energy phosphate ratios in the myocardium

of men with diabetes mellitus type I. *J Cardiovasc Magn Reson*, 4(4), 493-502 (2002)

59. G. N. Shivu, T. T. Phan, K. Abozguia, I. Ahmed, A. Wagenmakers, A. Henning, P. Narendran, M. Stevens and M. Frenneaux: Relationship between coronary microvascular dysfunction and cardiac energetics impairment in type 1 diabetes mellitus. *Circulation*, 121(10), 1209-15

60. R. van der Meer, S. Hammer, H. Lamb, M. Frolich, M. Diamant, L. Rijzewijk, A. de Roos, J. Romin and J. Smit: Effects of short-term high-fat, high energy diet on hepatic and myocardial triglyceride content in healthy men. *J Clin Endocrinol Metab*, 93(7), 2702-2708 (2008)

61. L. J. Rijzewijk, R. W. van der Meer, J. W. Smit, M. Diamant, J. J. Bax, S. Hammer, J. A. Romijn, A. de Roos and H. J. Lamb: Myocardial steatosis is an independent predictor of diastolic dysfunction in type 2 diabetes mellitus. *J Am Coll Cardiol*, 52(22), 1793-9 (2008)

62. S. Hammer, M. Snel, H. J. Lamb, I. M. Jazet, R. W. van der Meer, H. Pijl, E. A. Meinders, J. A. Romijn, A. de Roos and J. W. Smit: Prolonged caloric restriction in obese patients with type 2 diabetes mellitus decreases myocardial triglyceride content and improves myocardial function. *J Am Coll Cardiol*, 52(12), 1006-12 (2008)

63. J. O. Van Dobbenburgh, J. R. Lahpor, S. R. Woolley, N. de Jonge, C. Klopping and C. J. Van Echteld: Functional recovery after human heart transplantation is related to the metabolic condition of the hypothermic donor heart. *Circulation*, 94(11), 2831-6 (1996)

64. C. E. Haug, J. I. Shapiro, L. Chan and R. Weil, 3rd: P-31 nuclear magnetic resonance spectroscopic evaluation of heterotopic cardiac allograft rejection in the rat. *Transplantation*, 44(2), 175-8 (1987)

65. C. D. Fraser, Jr., V. P. Chacko, W. E. Jacobus, P. Mueller, R. L. Soulen, G. M. Hutchins, B. A. Reitz and W. A. Baumgartner: Early phosphorus 31 nuclear magnetic resonance bioenergetic changes potentially predict rejection in heterotopic cardiac allografts. *J Heart Transplant*, 9(3 Pt 1), 197-204 (1990)

66. G. J. Bruinsma, M. G. Nederhoff, C. W. van de Kolk, M. C. de Groot, P. J. Slootweg, J. J. Bredee, T. J. Ruigrok and C. J. Van Echteld: Bio-energetic response of the heart to dopamine following brain death-related reduced myocardial workload: a phosphorus-31 magnetic resonance spectroscopy study in the cat. *J Heart Lung Transplant*, 18(12), 1189-97 (1999)

67. P. A. Bottomley, R. G. Weiss, C. J. Hardy and W. A. Baumgartner: Myocardial high-energy phosphate metabolism and allograft rejection in patients with heart transplants. *Radiology*, 181(1), 67-75 (1991)

68. W. T. Evanochko, S. D. Buchthal, J. A. den Hollander, C. R. Katholi, R. C. Bourge, R. L. Benza, J. K. Kirklin and G. M. Pohost: Cardiac transplant patients response to the (31)P MRS stress test. *J Heart Lung Transplant*, 21(5), 522-9 (2002)

69. G. Fragasso, G. Perseghin, F. De Cobelli, A. Esposito, A. Palloshi, G. Lattuada, P. Scifo, G. Calori, A. Del Maschio and A. Margonato: Effects of metabolic modulation by trimetazidine on left ventricular function and phosphocreatine/adenosine triphosphate ratio in patients with heart failure. *Eur Heart J*, 27(8), 942-8 (2006)

70. D. J. Tyler, Y. Emmanuel, L. E. Cochlin, L. E. Hudsmith, C. J. Holloway, S. Neubauer, K. Clarke and M. D. Robson: Reproducibility of (31)P cardiac magnetic resonance spectroscopy at 3 T. *NMR Biomed* (2008)

71. P. A. Bottomley and C. J. Hardy: Proton Overhauser enhancements in human cardiac phosphorus NMR spectroscopy at 1.5 T. *Magn Reson Med*, 24(2), 384-90 (1992)

Key Words: Cardiac Magnetic Resonance Spectroscopy, Heart Failure, Cardiac Energy Metabolism, Review

Send correspondence to: Cameron Holloway, University of Oxford Centre for Clinical Magnetic Resonance (OCMR), Level 0, John Radcliffe Hospital, Oxford OX3 9DU, United Kingdom, Tel: 44 1865 221869, Fax: 44 1865 221111, E-mail: Cameron.Holloway@dpag.ox.ac.uk

http://www.bioscience.org/current/volS3.htm