

## Nuclear magnetic resonance spectroscopy: a tool for skeletal muscle metabolic research

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Nuclear magnetic resonance spectroscopy provides non-invasive and non-destructive access to the chemistry of human tissue *in situ*. Two studies presented in this issue of the Journal [1, 2] apply this new technique on skeletal muscle metabolism in patients with chronic lung disease.

In the 1940s, E. Purcell and F. Bloch discovered that electromagnetic radio-frequency energy could be used to interact with the magnetic fields of some atomic nuclei. The phenomenon was called nuclear magnetic resonance (NMR). For this discovery, Purcell and Bloch were awarded the Nobel Prize in 1952. In the 1950s, NMR was developed into NMR spectroscopy, as an instrument for analytical chemistry *in vitro*. The 1991 Nobel Prize in chemistry was awarded to R.R. Ernst for pioneering further development of the NMR technique, especially high resolution two dimensional spectroscopy.

The NMR-principle also proved usable for producing two or three dimensional representations of the proton density in tissue, thereby adding a novel technique for medical imaging called magnetic resonance imaging, (MRI). Later, the principle was also developed for spectroscopy *in vivo*, magnetic resonance spectroscopy (MRS). MRS in particular requires magnets with large field strength. By development of the super-conducting principle, strong magnets large enough to accommodate the human body have been constructed.

### Effects of atomic nuclear spin

Atomic nuclei spin around an axis. Since they are electrically charged, the spinning motion creates a magnetic momentum. If the nuclei are introduced into an external magnetic field, their magnetic momenta will tend to align themselves with that field. Nuclei like  $^1\text{H}$ ,  $^{13}\text{C}$ ,  $^{19}\text{F}$  and  $^{31}\text{P}$  can adopt only one of two possible orientations - alignment parallel or anti-parallel to the external magnetic field.

The individual nuclei do not align exactly parallel or anti-parallel with the external field but at an angle. Due to the magnetic momentum the nuclei precess (rotate) rapidly around the axis of the external magnetic field

in such a way that the spin-axis of the nuclei defines the surface of a cone. This precessional frequency ( $f$ ) is called the Larmor frequency and is expressed as:

$$f = \gamma B_0$$

where  $B_0$  is the strength of the external magnetic field and  $\gamma$  is related to the nucleus.

If a great number of nuclei with their spin axes randomly distributed on the surface of the imagined cone are considered, the components perpendicular to the external field will cancel out, and the net effect will be an orientation parallel or anti-parallel to the external field. These two orientations in the magnetic field represent different quantum energy states. By application of electromagnetic energy at a specific resonant frequency, the Larmor frequency, the nuclei may be forced to make transitions from the low energy state to the higher energy state. When this radiofrequency field (RF) is turned off, the nuclei will emit the absorbed energy as they return to the low energy state. This, too, is electromagnetic energy at the Larmor frequency - the magnetic resonance (MR) signal.

The Larmor equation is essential for the understanding of how information is gained for MRI as well as for MRS. During the excitation, specific nuclei can be selected by singling out proper values for  $f$  and  $B_0$  in relation to  $\gamma$  for these nuclei. In addition, a spatial selection of nuclei in one dimension can be obtained by modifying the main external magnetic field in such a way that a magnetic field gradient (change of strength per unit distance) is obtained in the desired direction.

### Magnetic resonance imaging (MRI)

Image information is the amplitude of the MR signal arising from various locations within an excited slice when the RF excitation is switched off. To encode these locations (*i.e.* the two remaining dimensions), processes called frequency and phase encoding, respectively, are applied. These processes also utilize the Larmor equation and variations of the magnetic field - but now during the emission phase. For frequency encoding a magnetic field gradient parallel to the plane of the slice is applied, and for phase encoding repeated measurements at varying field strengths have to be carried out.

For MRI the proton ( $^1\text{H}$ ) is the nucleus of choice, since it holds a strong magnetic moment which gives good detection sensitivity and has a high biological occurrence. By applying the magnetic field gradient technique during the RF excitation, protons localized in different slices perpendicular to the gradient axis can be excited selectively.

### Magnetic resonance spectroscopy (MRS)

MRS relies on the fact that the magnetic field influencing a nucleus is built up not only by the external field but also by components from magnetic nuclei, *i.e.* the molecular environment. Hence, in a homogeneous magnetic field, nuclei of the same elemental type will resonate at different frequencies, depending on the molecule of which it is a part. Thus, a spectrum can be obtained, enabling chemical assay. The minor differences in resonant frequency (*i.e.* the x-axis in the spectrum) are termed the "chemical shift" (CS). This shift is expressed as the relative change in strength of the magnetic field (ppm, parts per million) which would induce a corresponding change in resonant frequency.

The chemical shift for inorganic phosphate (Pi) relative to phosphocreatine (PCr) is 5 ppm. From this and the Larmor equation it is obvious that the magnetic field has to be extremely homogeneous (one in  $10^7$  or better) for MRS. The homogeneity of the magnetic field is corrected before each investigation by a procedure called "shimming".

The concentrations of protons in most tissues are of the order of 50–100 mol·kg<sup>-1</sup>, while the concentration of  $^{31}\text{P}$  as PCr is about 30 mmol·kg<sup>-1</sup>, *i.e.* about one to 2,000 times that of  $^1\text{H}$ . In addition, the  $^1\text{H}$  signal is much stronger than that from  $^{31}\text{P}$  at equimolar concentrations, due to differences in the magnetic moment. For these reasons, the tissue volume selected for a  $^{31}\text{P}$ -MR spectrogram must be some ml, *i.e.* much greater than the three dimensional resolution at  $^1\text{H}$ -MRI. By summing spectra acquired from a great number of excitations an adequate signal-to-noise ratio can be obtained.

Other nuclei such as  $^{13}\text{C}$  and  $^1\text{H}$  may also be used for *in vivo* MRS, making it possible to study, for instance, glucose, glycogen and lactate metabolism.

### Analysis

One problem with MRS *in vivo* is the quantification in absolute terms of the substances which have been detected. The tissue sample cannot be demarcated exactly and, therefore, the volume from which the signal is collected is not accurately known. Furthermore, the strength of the magnetic field and the absolute signal may vary over time. To avoid these problems, the levels of phosphate metabolites are commonly expressed in relative terms as fractions of the total intracellular phosphate pool.

The time resolution of MRS is slow compared with some biological events of interest to study, *e.g.* transformations of high energy phosphates at commencement or termination of muscle exercise. The relatively poor time resolution is due to the need to sum measurements from several excitations in order to reduce the signal-to-noise ratio, and to a saturation phenomenon. Saturation arises from the need for nuclear relaxation after one excitation before the next RF pulse can be discharged. The half time for relaxation is approximately 0.5 s in muscle tissue, which means that excitation intervals of about 3 s are appropriate. Averaging of at least twenty excitations means acquisition times of one minute or more.

The MR signal received after excitation is the sum of a large number of signals with slightly varying frequencies, corresponding to the resonant frequencies of nuclei at different localizations in gradient magnetic fields as in MRI, or of nuclei in different molecular environments as in MRS. The different frequency components of the combined signal are separated by Fourier transformation, giving amplitude as a function of frequency. The amplitude is proportional to the number of nuclei resonating at a particular frequency.

For MRI these data are subjected to further processing for image construction. For MRS the data are presented as a diagram - the MR-spectrogram (fig. 1).

### Application in energy metabolism

The list of analutes investigated by MRS is quite long. High energy phosphates - adenosine triphosphate (ATP), adenosine diphosphate (ADP), adenosine monophosphate (AMP), PCr, Pi, the nicotinamide-adenine dinucleotide (NADH), phosphodiester and monophosphate esters - are measured from  $^{31}\text{P}$  spectra.  $^{31}\text{P}$ -MRS has also made it possible to study exchange rates among Pi, PCr, ADP and ATP [3]. The decrease in PCr and the concomitant rise in Pi provide an index (PCr/Pi ratio) of cellular energy state.

$^{31}\text{P}$ -MRS can also indirectly measure intracellular pH, since the chemical shift for cellular Pi vs PCr on a  $^{31}\text{P}$ -MR spectrum is pH dependent [4]. This phenomenon makes it possible to indirectly monitor glycolytic activity in the tissue sample studied.

Reports of muscle biochemistry appeared in the middle of the 1970s [5]. Early studies of human skeletal muscle metabolism with MRS were performed in legs and arms, because they were easily placed in the magnets. The effects of myopathies [6], dystrophies [7], muscle injury [8] and exercise [9] on muscle metabolism were studied by analysis of changes in high energy phosphates, Pi and pH.

Spectra obtained at rest can be markers of disease, as in Duchenne dystrophy in which PCr, Pi and pH are abnormal at rest [7]. However, to distinguish diseased from normal muscle tissue, exercise has often been used [9, 10]. Analysis then includes the degree and rate of fall in PCr and pH, rise in Pi during

exercise, and rate of reversal during recovery. Reduced PCr/Pi and PCr/ATP ratios and elevated Pi at rest, as well as slower recovery of metabolites, prolonged acidosis, or elevated or neutral pH during exercise and recovery, are found not only in muscle enzyme deficiencies, but also in mitochondrial myopathies [11], post viral exhaustion syndrome [12] and malignant hyperthermia susceptibility [13].

Functional impairment due to cardiovascular disease produces changes in skeletal muscle metabolism. In congestive heart failure, the Pi value is generally elevated before exercise, the PCr value and pH fall more rapidly and to a greater extent during exercise, and the pH recovers more slowly [14, 15]. In occlusive arterial disease of the legs, recovery rates of pH and PCr are reduced [16].

Reports of effects of lung disease on skeletal muscle metabolism using the MRS technique have been few. Up to now, studies have often been performed with analysis of metabolites from samples of skeletal muscle usually obtained by needle biopsy. Using MRS the same tissue sample can now be studied repeatedly. This has made possible sequential studies of the effect of different levels of hypoxia on muscle energy state. In an animal study on isolated skeletal muscle, PCr declined and Pi increased when arterial oxygen tension ( $P_{O_2}$ ) fell to levels below 40–50 mmHg (5.3–6.6 kPa) [17]. In another study on isolated skeletal muscle, total ischaemia was tolerated better than hypoxaemia, as indicated by a decreased rate of PCr recovery following hypoxaemia compared to ischaemia [18]. This study indicated that factors other than lack of oxygen modulate cellular response to hypoxia.  $^{31}\text{P}$ -MRS has made it possible to study the metabolic properties of slow and fast twitch muscle fibres simultaneously [19].

Unfortunately the study of respiratory muscle *in vivo* with  $^{31}\text{P}$ -MRS is complicated by the motion of the thorax and diaphragm. To minimize the artifacts of ventilation movements, gating techniques may be used.

In this issue of the Journal WUYAM *et al.* [1] and TADA *et al.* [2] present two of the first studies of the effect of chronic obstructive pulmonary disease (COPD) on skeletal muscle metabolism using  $^{31}\text{P}$ -MRS. The first study shows increased Pi/PCr ratio for COPD patients compared to normals during exercise, as well as decreased pH. In both studies the resynthesis rate of PCr during recovery was slower in COPD patients than the controls. The results of these two studies suggest impairment of aerobic capacity in nonventilatory skeletal muscle during exercise in chronic hypoxaemia.

Although most studies of skeletal muscle metabolism with MRS have used  $^{31}\text{P}$  spectroscopy, there are also reports of both  $^{13}\text{C}$  and  $^1\text{H}$  spectrum analysis. With  $^{13}\text{C}$ -MRS, quantification of muscle glycogen synthesis is possible [20]. Using compounds labelled with  $^{13}\text{C}$ , fluxes through metabolic pathways such as the tricarboxylic acid cycle can be studied [21].  $^1\text{H}$  MRS can detect triglycerides. In Duchenne muscular dystrophy, replacement of muscle by fat has been found [7].

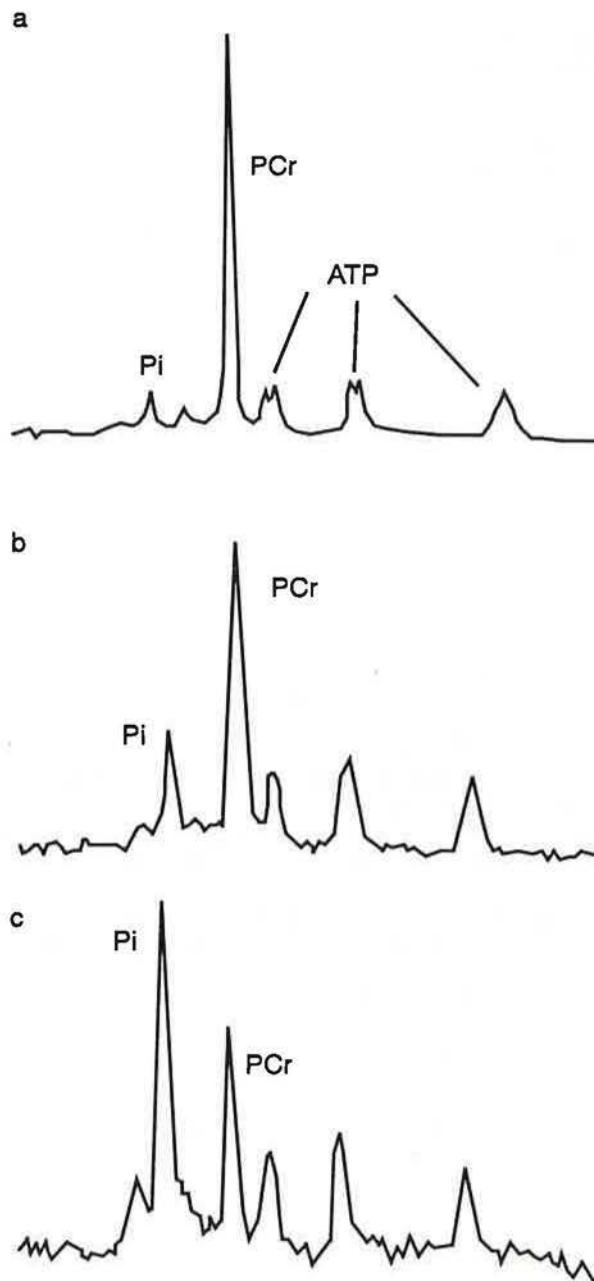


Fig. 1. -  $^{31}\text{P}$  NMR spectra of gastrocnemius muscle at rest and during exercise. a) Spectrum of gastrocnemius muscle at rest. The tall peak is PCr. The three peaks upfield (to the right) of PCr are the  $\gamma$ -,  $\alpha$ - and  $\beta$ -peaks of ATP. Pi is downfield (to the left) of PCr. Resting spectra were similar in patients with heart failure and control subjects. b) Spectrum from a control subject. c) Spectrum from a patient with heart failure during exercise at 1.5 W. PCr is lower and Pi is higher in the patient with heart failure. NMR: nuclear magnetic resonance; PCr: phosphocreatine; ATP: adenosine triphosphate; Pi: inorganic phosphate. (Reproduced with permission, from Clinical Science and L. Arnolda *et al.* [22]).

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