Introduction to in vivo 31P magnetic resonance spectroscopy of (human) skeletal muscle

A. Heerschap1*, C. Houtman2, H. J. A. in 't Zandt1, A. J. van den Bergh1 and B. Wieringa3

1Department of Radiology, 2Department of Neurology, 3Department of Cell Biology and Histology, Faculty of Medical Sciences, University of Nijmegen, 6500 HB Nijmegen, The Netherlands

31P magnetic resonance spectroscopy (MRS) offers a unique non-invasive window on energy metabolism in skeletal muscle, with possibilities for longitudinal studies and of obtaining important bioenergetic data continuously and with sufficient time resolution during muscle exercise. The present paper provides an introductory overview of the current status of in vivo 31P MRS of skeletal muscle, focusing on human applications, but with some illustrative examples from studies on transgenic mice. Topics which are described in the present paper are the information content of the 31P magnetic resonance spectrum of skeletal muscle, some practical issues in the performance of this MRS methodology, related muscle biochemistry and the validity of interpreting results in terms of biochemical processes, the possibility of investigating reaction kinetics in vivo and some indications for fibre-type heterogeneity as seen in spectra obtained during exercise.

31P magnetic resonance spectroscopy: Skeletal muscle: High-energy phosphates: Energy metabolism: Exercise

Following initial experiments on animal tissue (Hoult et al., 1974; Ackerman et al., 1980), magnetic resonance (MR) spectroscopy (MRS) was first applied to human subjects in the early 1980s, using the 31P nucleus to monitor the levels and fate of high-energy phosphates in skeletal muscle (Chance et al., 1981; Cresshull et al., 1981; Ross et al., 1981). From these first experiments it was clear that 31P MRS offers a unique non-invasive window on energy metabolism in skeletal muscle. Of particular interest is the possibility of obtaining important bioenergetic data continuously and with sufficient time resolution during muscle exercise. Another important aspect is that longitudinal monitoring is possible. Numerous studies applying this technique to human subjects have been published, and several reviews are available addressing specific results obtained in this way (for example, see Barbiroli, 1992; Cozzone & Bendahan, 1994; Kemp & Radda, 1994; McCully et al., 1994; Radda et al., 1995).

The present paper provides an introduction to 31P MRS as applied to skeletal muscle of human subjects, and also gives some illustrative examples from our recent studies on skeletal muscle of transgenic mouse models lacking creatine kinase (EC 2.7.3.2).

Information content of the 31P magnetic resonance spectrum of skeletal muscle

To appreciate the potential of the method, first, the information content of a spectrum obtained from human skeletal muscle at rest should be examined (see Fig. 1(A)). The most dominant signals in the spectrum are from phosphocreatine (PCr) and the three non-equivalent phosphate groups of ATP. Usually also a signal for inorganic phosphate (Pi) can be observed, and under favourable conditions signals for phosphomonoesters and phosphodiesters are observable as well. What is the origin of the distinct resonance frequencies of the 31P nuclei in these compounds? In a first approximation the resonance frequency of the nuclear spins is determined by the main magnetic field. In addition this main field interacts with the electronic environment of the nuclei, inducing small counter magnetic fields. The strength of the counter field at each nucleus depends on its chemical environment. This factor has an important consequence, i.e. nuclei within different chemical structures acquire different resonance frequencies. As a result of this chemically-related frequency separation of phosphate resonances, the spectrum provides a type of

Abbreviations: MR, magnetic resonance; MRS, MR spectroscopy; PCr, phosphocreatine; Pi, inorganic phosphate.
*Corresponding author: Dr A. Heerschap, fax +31 24 3540 866, email a.heerschap@rdiag.azn.nl
fingerprint of the phosphate chemical content of the muscle. For convenience the relative resonance frequencies, or chemical shifts, are expressed with respect to the resonance frequency of the main field in parts per 10⁶ (ppm). For in vivo 3¹P MRS the position of the PCr resonance is usually selected as a reference (i.e. 0 ppm).

A closer inspection of some resonances in the 3¹P MR spectrum of skeletal muscle may reveal some fine structure, which is due to their phosphate nuclear spins being subject to an interaction with other nearby spins, the so-called spin–spin coupling. This coupling may be with either neighbouring phosphate spins or proton spins. Phospho–phosphate spin coupling can be observed for the resonances of ATP as line splittings, i.e. the ATP signal becomes a triplet and the a, b and γ ATP signals become doublets (see Fig. 1(A)). Proton–phosphate spin couplings are effective for phosphomonoesters, phosphodiesters, ATP and diphosphodiesters resonances. In vivo these couplings are usually not resolved, but result in broadened lines.

As the chemical environment of the 3¹P nuclei may change with changes in the physiological condition of the muscle, the phosphate resonance positions may change as well. Of particular importance is the effect of pH variation on spectral resonance positions (Moon & Richards, 1973). This effect is demonstrated in the spectra shown in Fig. 2, which were obtained from a solution containing various biochemical compounds such as ATP, PCr and Pi at two different pH values. The frequency shift observed for the peak of Pi with its dissociation constant (pKₐ) of about 6-9 is physiologically most relevant. The position of the PCr peak remains constant. Thus, the Pi–PCr shift difference is used to derive pH values, applying the appropriate titration curves. As the signal for Pi in spectra for skeletal muscle arises from the sarcoplasm, under normal conditions, it is the intracellular pH that is determined in this way (see Gadian, 1995).

Another physiological condition that may affect resonance positions is the cellular Mg²⁺ content. In particular, the β ATP resonance frequency is sensitive to Mg²⁺ binding (Gupta & Moore, 1980). Recent studies based on calculations of the free cytosolic Mg²⁺ concentration in skeletal muscle from shifts in ATP resonances have revealed changes in this concentration as a result of exercise (Iotti et al. 1999) and pathology (Park et al. 1999).

3¹P MR spectra for individual skeletal muscles in normal adult human subjects obtained at rest are fairly reproducible, although some conditions may cause variations, e.g. training and oral supplementation with creatine may increase the MRS-visible phosphate content (Park et al. 1988; Kreis et al. 1996), strenuous exercise may increase the Pi : PCr value for some days (McCully et al. 1994) and the phosphodiester signal may be increased in older subjects (Sastrustegui et al. 1988). Significant changes are observed during postnatal development (Heerschap et al. 1988; McCully et al. 1994). A higher Pi : PCr and tendency to alkaline tissue pH is a common finding in the diseased state (see Heerschap et al. 1993; Cozzone & Bendahan, 1994). In mitochondrial dysfunction this change may be associated with increased ADP levels in the muscle at rest; however, metabolic abnormalities are better characterized in dynamic experiments performing 3¹P MRS during exercise and recovery (see later).
Some practical issues in the performance of $^{31}$P magnetic resonance spectroscopy with human muscle

**Hardware**

Current clinical MR systems, mostly operating at 1.5 T, are focused on the use of the $^1$H nucleus for the imaging of water in the body. To perform $^{31}$P MRS with these systems additional hard- and software is required, such as specialized radio frequency coils and for optimal performance a so-called second radio frequency channel. $^{31}$P MRS of human skeletal muscle is also performed using experimental MR systems up to field strengths of 4-7 T with limited space in the magnet bores, so that usually only the distal half of arms or legs can be studied. On most of these magnets a fixed horizontal position for the limb is required, while the clinical systems with larger magnet bores allow for more positional freedom. The major advantages of higher field strengths are the improvement in sensitivity and spectral resolution. This factor is one of the driving forces for installing higher-field magnets, and it can be expected that the application of *in vivo* $^{31}$P MRS to human muscle at still higher field strengths (7 or 8 T) will allow the study of more subtle and ill-understood features of phosphate metabolism in the future. Since dynamic experiments including muscle exercise are most informative, some effort has been put into the development of in-magnet exercise devices (ergometers) which obviously should be constructed without magnetic materials.

$^1$H decoupling

As described earlier, the spins of some $^{31}$P nuclei experience an interaction with nearby $^1$H spins causing line broadening. Using a so-called second radio frequency channel, it is possible to decouple this interaction by irradiation at the $^1$H frequency (Luyten et al. 1989). The line splittings collapse to give narrower and higher spectral lines, which improves spectral resolution and sensitivity. Additional peaks become resolved, such as those for phosphatidyl choline and phosphatidylethanolamine at the phosphodiester position (see Fig. 1B). On increasing the field strength the line widths of $^{31}$P resonances obtained *in vivo* become broader, and this effect starts to dominate the spin–spin coupling, which is independent of field strength. In practice decoupling above field strengths of about 4 T has little effect, but as the improvement in chemical shift separation brings more gain than the resolution loss caused by line broadening, such a higher field can be tolerated. The lower field combined with $^1$H decoupling.

Another useful effect is the so-called nuclear Overhauser effect, by which it is possible to 'pump' magnetization from the $^1$H nuclei to the $^{31}$P nuclei. Also, for this effect a second radio frequency channel may be useful. In this way the sensitivity of detecting $^{31}$P resonances at 1.5 T can be improved up to about 70 % (Bachert & Belleman, 1992; Brown et al. 1995).

**Localization, spatial and temporal resolution**

The possibility of obtaining $^{31}$P MR spectra from selected tissue areas, and the spatial and temporal resolution of $^{31}$P MRS are important issues to take into consideration in the design of experimental protocols. For $^{31}$P MRS of human muscle it is very common to employ a circular (or ellipsoid) radio frequency surface coil for signal excitation and reception. The dimensions of the coil are adapted to the muscle or muscle part of interest. Signals from the tissue adjacent to the coil are selectively sampled in this way from a hemisphere with a radius approximately equal to the radius of the coil. Although this approach to performing $^{31}$P MRS is convenient and sensitive, it is sometimes necessary to apply more precise localization of the tissue region from which the signal has to be sampled. Several methods have been developed for this purpose over the years. Currently, gradient-based methods for the selection of tissue volumes are used in which voxel location and dimensions can be set arbitrarily. This technique enables researchers and clinicians to zoom in on different subtypes of muscles if required. A popular single voxel technique for $^{31}$P MRS is a multi-acquisition-shot method called image-selected *in vivo* spectroscopy (ISIS; Ordidge et al. 1986). The minimum volume that can be selected with sufficient signal : noise depends on a number of factors, but as a 'rule of the thumb' it needs to be at least about 15 ml for $^{31}$P MRS at the field strength of 1.5 T. Multi-voxel localization may be provided by so-called spectroscopic imaging or chemical shift imaging methods (Brown, 1992). This technique can also be applied to human skeletal muscle, as illustrated by the example shown in Fig. 3.

In dynamic studies it is often important to optimize the time resolution of an experiment. As larger volumes of detection produce a better signal : noise value in the same measurement time, generally the largest possible volume that is assumed to be sufficiently homogeneous is selected in time-critical experiments. In dynamic $^{31}$P MRS of human skeletal muscle time resolution is usually between 1 min and 1 min.

**Sensitivity and magnetic resonance visibility in the detection of phosphorus metabolites**

As $^{31}$P nuclei are present at 100 % natural abundance in body compounds the question may arise as to why all phosphate compounds present in skeletal muscle are not visible in the MR spectrum. This situation is related to the sensitivity of the MRS method. At the presently-employed clinical field strengths and experimental conditions the phosphate concentration of a compound has to be more than 0.1 mmol/kg wet weight, and in most experiments a threshold concentration of 0.5 mmol/kg wet weight is required. Furthermore, only small mobile compounds generate resonances with sufficiently small line widths to make them visible. These restrictions limit the number of visible phosphate compounds in skeletal muscle to about eight. The determination of differences in glucose-6-phosphate levels in human muscle of less than 0.1 mmol/kg (Rothman et al. 1992) illustrate the current lower limits of *in vivo* $^{31}$P MRS measurements under optimal conditions.

Binding of small phosphate compounds to large subcellular structures or macromolecules limits their rotational freedom and renders them MR invisible. This limitation implies that with $^{31}$P MRS only the tissue contents
of free phosphate compounds are monitored, which is of interest as this measurement provides the relevant variables in the evaluation of biochemical processes in the cell. Invasive methods involving biopsies or freeze clamping of muscle tissue usually only estimate the total cellular level of a compound. Since quantitative analyses by both invasive methods and $^{31}$P MRS result in similar skeletal muscle contents for ATP, it is assumed that this compound is fully MRS visible in this tissue (Gadian, 1995). However, $^{31}$P MRS often gives a relatively higher level for PCr, and the difference has been attributed to the rapid breakdown mediated by the creatine kinase reaction during the freeze-clamping procedure (Meyer et al. 1982); therefore, the PCr level is also considered to be fully MRS visible. On the other hand, $^{31}$P MRS often gives lower levels for Pi than invasive methods, and therefore it is assumed that a significant proportion of this compound is immobilized in subcellular structures or bound to macromolecules (Gadian, 1995). More detailed studies suggest that immobilization in the mitochondrial matrix is the causative element underlying this difference (Hutson et al. 1992). Based on this knowledge, changes in the intensity of the Pi signal have been used to quantify the mitochondrial uptake of Pi (Iotti et al. 1996).

**Data processing**

Finally, appropriate quantitative evaluation of resonance integrals and chemical shifts in $^{31}$P MR spectra is of importance. This evaluation is most easily performed using special software developed for the purpose, such as the time domain analysis software developed by de Beer & van Ormondt (1992) and van den Boogaart (1997), which allows the incorporation of previous knowledge for improved spectral fitting. In spectroscopic imaging experiments or in dynamic experiments with repetitive measurements, large data sets may be produced which need some type of automatic processing, and methods such as those proposed by Stoyanova et al. (1995) and VanHamme et al. (1999) may be appropriate. Resonance integrals may be further evaluated as ratios. However, absolute quantities in terms of tissue or cellular contents of compounds can also be derived, provided that a proper reference is available and other variables such as signal saturation are known. Often ATP is taken as a reference, assuming a certain cellular ATP concentration at rest. This assumption seems legitimate, as tissue ATP appears relatively constant under most conditions. Also, tissue water has been introduced as an internal reference (Thulborn & Ackerman, 1983).

**Cellular and molecular conclusions from macroscopic measurements by $^{31}$P magnetic resonance spectroscopy**

It should be clear that in vivo $^{31}$P MRS essentially provides information on phosphate compounds at the tissue level. Individual muscle cells and intracellular microcompartments with different metabolite levels are not directly accessible using in vivo $^{31}$P MRS. However, evaluations and interpretations at a cellular or subcellular level are possible when certain assumptions can be made about the origin of the signals under observation. For instance, as the sarcoplasm in muscle is the cellular compartment which contains the bulk of the $^{31}$P MRS-visible compounds, it is the phosphate metabolism of this compartment that is principally observed, although metabolic processes in

---

**Fig. 3.** Two-dimensional $^{31}$P magnetic resonance spectroscopic imaging of the m. tibialis anterior (TA). The data have been obtained using an ellipsoid-shaped surface coil placed over the TA. The two hot spots on the limb surface in the images indicate the position of the coil (→). The pulse repetition time was 7 s. The voxel dimension was $15 \times 15 \text{ mm}$. In the sagittal direction localization was dictated by the surface coil length (200 mm). Only the spectral region including the phosphocreatine (PCr) and inorganic phosphate (Pi) peaks is plotted in the voxels. (A) Spectroscopic image obtained at rest. A dominant signal for PCr is visible only in the TA. (B) Spectroscopic image obtained during exercise. Now an increased Pi peak is observed at the left side of the PCr peak.
cell-organelle compartments, such as the mitochondria, may have profound effects on metabolism in the sarcomplasm. One complicating factor in data interpretation could be the presence of cellular heterogeneity. However, some compelling arguments have been put forward by Kushmerick (1995) in favour of the view that any human skeletal muscle can be considered as a unimodal continuum of properties of a relatively small range of cell types, from which it is concluded that, overall, bioenergetic variables measured by $^{31}$P MRS for a given skeletal muscle can be interpreted in terms of biochemical and molecular mechanisms. A further complicating factor in the interpretation of data could be metabolic compartmentalization within the cytoplasmic domain of the muscle cell. Specifically designed experiments by Wiseman & Kushmerick (1995) appear to indicate that in skeletal muscle the physico-chemical properties of bulk cytoplasm are not influenced by possible subcompartments with different ATP or ADP contents, and that the creatine kinase reaction observed by $^{31}$P MRS can be most easily understood in terms of solution thermodynamics, with all substrates of creatine kinase being freely available to the enzyme. However, this viewpoint, in which cellular interpretation of $^{31}$P MRS data for muscle does not consider the existence of metabolic micro-environments and subcellular structures, has been criticized (Walliman, 1996).

**Muscle bioenergetics as observed by dynamic $^{31}$P magnetic resonance spectroscopy**

The immediate source of free energy for energy-consuming processes in the muscle comes from the reaction:

$$\text{ATP} + \text{H}_2\text{O} \rightarrow \text{ADP} + \text{Pi} + \text{H}^+.$$  \hspace{1cm} (1)

During a contractile event this reaction is catalysed mainly by the enzyme myosin ATPase (EC 2.7.1.117) and by ATPases involved in Ca homeostasis. The ATP pool in the muscle is only sufficient for very brief periods of contractile activity, and therefore energy has to be provided by other pathways. A readily-available back-up reserve is present in the form of the high-energy phosphate compound PCr. The enzyme creatine kinase catalyses the reversible reaction:

$$\text{PCr} + \text{ADP} + \text{H}^+ \leftrightarrow \text{ATP} + \text{Cr},$$  \hspace{1cm} (2)

and thereby enables the pool of high-energy phosphate groups present in ATP to be replenished by that in PCr. This reaction occurs at different cellular locations, both in the cytosol and in the mitochondria, where it is mediated by different members of the creatine kinase family.

Combination of equations 1 and 2 gives:

$$\text{PCr} + \text{H}_2\text{O} \rightarrow \text{Cr} + \text{Pi}.$$  \hspace{1cm} (3)

When ATP levels are maintained at a constant level during energy consumption, a decrease in PCr levels should be accompanied by a stoichiometric increase of Pi levels. $^{31}$P MRS provides the most direct evidence that this is indeed what happens, as illustrated in a stack plot of spectra obtained sequentially during exercise (see Fig. 4). More detailed analyses of such experiments often shows some subtle differences from this simple viewpoint, e.g. some breakdown of ATP and some uncompensated loss of Pi signal intensity may occur.

Furthermore, PCr levels are only sufficient for a limited period of muscle activity, and would rapidly be depleted in the absence of further energy sources and/or mechanisms for vectorial transport of high-energy phosphate groups. Under aerobic conditions mitochondrial respiration serves as the major source of high-energy phosphate groups such as ATP and PCr. In addition to having a role in balancing ATP levels, the PCr–creatine kinase system is considered to be an efficient intermediate in the transfer of high-energy phosphates from the sites of energy production (mitochondria, glycolytic loci) to the energy-consuming locations in the muscle cell, a potential function which has attracted considerable attention (Bessman & Geiger, 1981; Meyer et al. 1984; Walliman et al. 1992; Saks & Ventura-Clapier, 1994). Under anaerobic conditions, when glycolysis is left as the main energy-generating process, the role of the PCr circuit as a back-up system for preserving the integrity of energy homeostasis becomes clearly evident. Some direct evidence for this role has come from experiments with skeletal muscle of transgenic mice that were genetically depleted of the enzyme creatine kinase (In ’t Zandt et al. 1999a). During an ischaemic period the ATP levels decrease in these mice, while they are maintained in control mice (see Fig. 5(A)).

The reaction scheme shown in equation 2 suggests that the creatine kinase reaction may also function in stabilizing pH during contraction or hypoxia–ischaemia. This function was indeed apparent in the ischaemia experiments with the creatine kinase-deficient mice described earlier. In the deficient mice the pH decreased to much lower values than those in control mice. Also, the pH undershoot observed in


Free ADP is at too low a level in the muscle cell to be assessed experimentally by direct measurements using \(^{31}\)P MRS studies (for example, see In ‘t Zandt et al. 1999b; Kreis et al. 1999; Kruiskamp & Nicolay, 1999). ADP concentrations calculated in this way resulted in much lower values than those derived by chemical methods after freeze clamping, which makes sense as in the latter methods total ADP levels (free and bound) are obtained. In a similar way global free AMP concentrations may be estimated from the equation:

\[
\text{AMP} = \frac{\text{(ADP)}^2}{k \times \text{ATP}}.
\]

Thus, a number of essential variables obtained either directly or indirectly from dynamic \(^{31}\)P MRS can be used for quantitative studies of the kinetics of energy metabolism \textit{in vivo}. In this manner it is possible to obtain reliable estimates of the rates of aerobic and anaerobic ATP synthesis, and the influence of metabolic control. This information is the major contribution that \(^{31}\)P MRS can offer to the field of physiology and metabolism of human skeletal muscle. At present no other technique can match this potential for longitudinal non-invasive metabolic investigations of the intact living muscle.

As an example, a central issue in \(^{31}\)P MRS of skeletal muscle has been the metabolic control of mitochondrial function. Several approaches have been followed; for instance, Chance et al. (1986) have used a graded steady-state non-exhaustive exercise protocol to identify ADP as a principal control element of oxidative metabolism in human skeletal muscle under these conditions. In this study a Michaelis–Menton type of equation yielded an apparent maximum velocity as a measure of oxidative capacity. Other workers (for example, see Taylor et al. 1983, 1986; Bendahan et al. 1990; Barbiroli, 1992; Radda et al. 1995) have measured the rate of oxidative metabolism under non-steady-state exercise conditions. In particular, the recovery rate of PCr immediately after submaximal exercise appears to reflect mitochondrial capacity (Lodi et al. 1997). No recovery of the PCr content occurs during ischaemia after exercise (Taylor et al. 1983; Quistorff et al. 1993).

Thus, to confirm qualitative information, quantitative assessment is becoming increasingly important. A comprehensive method for the quantitative interpretation of \(^{31}\)P MRS measurements of muscle before, during and after several types of muscle exercise has been presented by Kemp & Radda (1994). With this method it is possible to estimate rates of glycogenolytic and aerobic ATP synthesis during exercise, as well as the magnitude of oxidative ATP synthesis and proton efflux during recovery from exercise. The oxidative capacity associated with steady-state exercise can be assessed quantitatively. An improved method of assessing ADP recovery after exercise has recently been reported (Chen et al. 1999). Results from MRS studies of human skeletal muscle have also been interpreted in terms

---

The oxidative capacity associated with steady-state exercise can be assessed quantitatively. An improved method of assessing ADP recovery after exercise has recently been reported (Chen et al. 1999). Results from MRS studies of human skeletal muscle have also been interpreted in terms of energy metabolism and metabolic control. This information is the major contribution that \(^{31}\)P MRS can offer to the field of physiology and metabolism of human skeletal muscle. At present no other technique can match this potential for longitudinal non-invasive metabolic investigations of the intact living muscle.

As an example, a central issue in \(^{31}\)P MRS of skeletal muscle has been the metabolic control of mitochondrial function. Several approaches have been followed; for instance, Chance et al. (1986) have used a graded steady-state non-exhaustive exercise protocol to identify ADP as a principal control element of oxidative metabolism in human skeletal muscle under these conditions. In this study a Michaelis–Menton type of equation yielded an apparent maximum velocity as a measure of oxidative capacity. Other workers (for example, see Taylor et al. 1983, 1986; Bendahan et al. 1990; Barbiroli, 1992; Radda et al. 1995) have measured the rate of oxidative metabolism under non-steady-state exercise conditions. In particular, the recovery rate of PCr immediately after submaximal exercise appears to reflect mitochondrial capacity (Lodi et al. 1997). No recovery of the PCr content occurs during ischaemia after exercise (Taylor et al. 1983; Quistorff et al. 1993).

Thus, to confirm qualitative information, quantitative assessment is becoming increasingly important. A comprehensive method for the quantitative interpretation of \(^{31}\)P MRS measurements of muscle before, during and after several types of muscle exercise has been presented by Kemp & Radda (1994). With this method it is possible to estimate rates of glycogenolytic and aerobic ATP synthesis during exercise, as well as the magnitude of oxidative ATP synthesis and proton efflux during recovery from exercise. The oxidative capacity associated with steady-state exercise can be assessed quantitatively. An improved method of assessing ADP recovery after exercise has recently been reported (Chen et al. 1999). Results from MRS studies of human skeletal muscle have also been interpreted in terms
of metabolic control analysis (Shulman et al. 1995) or metabolic control theory (Jenesson et al. 1999).

Importantly, in either a more qualitative or a quantitative sense, $^{31}$P MRS approaches have also been used to characterize various conditions of human muscle energy metabolism, e.g. in diseased muscle, injured muscle, fatigued muscle, muscle adapted to increased (dis)use, etc. (for example, see Ross et al. 1981; Duboc et al. 1987; deGroot et al. 1993; Cozzone & Bendahan, 1994; McCully et al. 1994; Radda et al. 1995).

Combinations of $^{31}$P MR measurements with other MR measurements (e.g. $^{1}$H, $^{13}$C) in the same measurement session or experimental setting may offer a powerful extended view of metabolic and physiological processes in skeletal muscle, e.g. as demonstrated in studies of diabetes mellitus (Shulman et al. 1996).

**Chemical reaction kinetics in vivo by $^{31}$P magnetization transfer**

$^{31}$P MRS offers a unique possibility to determine flux rates in biochemical pathways in vivo by the magnetization transfer method (Brindle, 1988; Rudin & Sauter, 1992). The technique involves perturbation of the magnetization of a nuclear spin system in a particular compound, and monitoring how this perturbation influences the nuclear magnetization of this spin system present in another compound with which it is in chemical exchange. This process is illustrated by the spectra in Fig. 6 (A–C) for wild-type mouse skeletal muscle. Fig. 6(A) is a control spectrum, while for the spectrum in Fig. 6(B) the $\gamma$-phosphate spin of ATP was selectively irradiated to make its signal disappear.

![Fig. 6. Saturation transfer experiment using mouse skeletal muscle. (A–C), Spectra obtained from the hindlimb of a wild-type mouse; (D–F), spectra from a creatine kinase-deficient mouse. (A, D), Control spectra; (B, E), spectra obtained with saturation of the $\gamma$ ATP (↓); (C, F), the difference spectra showing an effect on phosphocreatine (PCr) in the case of wild-type mice and the absence of an effect for creatine kinase-deficient muscle. ppm, Parts per 10$^6$.](https://www.cambridge.org/core/terms).

Fig. 6(C) shows the difference spectrum, and it is immediately clear that part of the PCr signal has disappeared as well, which is because it is in rapid exchange with the $\gamma$-phosphate of ATP in the creatine kinase reaction. Magnetization transfer in this type of experiment is commonly called saturation transfer. From a series of such experiments it is possible to estimate the rate-constants of the creatine kinase reaction, and thus the flux through this reaction. Reaction rates of between about 0·05 and 10/s can be estimated by this method (Meyer, 1982; Rudin & Sauter, 1992). We also performed a saturation transfer experiment using mouse skeletal muscle deficient in creatine kinase (see Fig. 6(D–F)). Irradiating the $\gamma$-phosphate peak had no effect on the PCr signal (Fig. 6(F)), a clear demonstration that the magnitude of exchange had decreased below MRS detection. In these mice PCr was found to be almost metabolically inactive (Steeghs et al. 1997; In ’t Zandt et al. 1999a).

This technique has also been applied to human skeletal muscle (for example, see Rees et al. 1989). Both animal and human studies are in agreement with the notion that in skeletal muscle at rest the creatine kinase reaction is in equilibrium, and that the flux through this reaction is faster than the net ATP turnover rates. Contrary to expectation, in two studies on human subjects the flux from PCr to ATP decreased during muscle exercise (Rees et al. 1989; Goudemant et al. 1997). However, a recent study showed that this flux was increased or similar to resting levels during exercise (Horska et al. 1999).

**Heterogeneity in muscle pH during exercise**

In general $^{31}$P MR spectra of resting human skeletal muscle appear to be relatively uniform, in agreement with the viewpoint of Kushmerick (1995), although some studies indicate small variations in the Pi:PCr value, possibly related to fast- and slow-twitch fibre composition of the muscle studied (VandenBorne et al. 1995). During exercise more than one Pi peak may appear in the spectra at different positions, and this pH heterogeneity has been taken as a reflection of different fibre types in the muscle under study (Park et al. 1987): i.e. the glycolytic fibres show faster acidification during exercise than the oxidative fibres. An alternative explanation for this observation was that the surface coil used in these experiments views more than one muscle type; e.g. two muscles, of which one is more heavily involved in exercise than the other. However, further experiments indicated that these different Pi peaks may arise from within a single muscle (VandenBorne et al. 1993; Mizuno et al. 1994). We have explored this phenomenon in m. tibialis anterior, a muscle without synergistic partners, thus avoiding some complications in the interpretation of this phenomenon. Using chemical shift imaging during steady-state exercise we have observed a double Pi peak in voxels completely located in this muscle (see Fig. 7), thus confirming observations on other muscles (Park et al. 1987; Achten et al. 1990; VandenBorne et al. 1993; Mizuno et al. 1994). Interestingly, some voxels in the m. tibialis anterior only show one increased Pi peak, indicating that in these parts of the muscle energy production is more homogenous.
Acknowledgements

We thank Mark Rijpkema for obtaining the $^{31}$P MR spectrum of human skeletal muscle at rest. We also thank H. van den Boogert for excellent technical assistance.

References


Fig. 7. Splitting of the inorganic phosphate (Pi) peak in exercising muscle. Selected spectra are from voxels in the m. tibialis anterior obtained by the chemical shift imaging experiment shown in Fig. 3. Only the spectral regions including the phosphocreatine (PCr) and inorganic phosphate (Pi) peak are displayed. pH values are indicated. (---), The spectra obtained at rest; (—), the spectra obtained during exercise. The Pi peak has increased in all voxels. The medial part of the m. tibialis anterior shows split Pi peaks (A,B,D), while in the lateral part only one Pi peak occurs at near neutral pH (C,E). ppm, Parts per 10^6.


