

P-31 MRS of muscle diseases

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Thirty years ago, Hoult *et al.* reported for the first time that phosphorus metabolites could be observed *in vivo* using ^{31}P Magnetic Resonance Spectroscopy (MRS) opening promising opportunities of understanding muscle energetics *in vivo* under strictly non invasive conditions [1]. From that time, MRS technology has rapidly evolved with the development of RF-surface coils in 1980 [2], the availability of high field and wide-bore superconducting magnets and methodological developments (dedicated pulse sequences, spatial localization of NMR signal...). So far, a large number of publications has been devoted to the investigation of muscle energetics in a variety of conditions ranging from diseases [3-5] to training regimens [6-8]. Considering the investigation of muscle diseases using ^{31}P MRS, the topic can be approached in different ways. The classical way is to list the number of pathologies investigated so far and to present the corresponding metabolic anomalies. From our point of view, this way suffers from the lack of a critical approach. In that respect, we have chosen in this syllabus to start with an overview of the possibilities offered by the technique (paragraphs I and II). Paragraph III is devoted to a presentation of muscle energetics and the corresponding changes occurring in exercising muscle as seen using ^{31}P MRS are presented in paragraph IV. The reader should then have acquired sufficient knowledge to properly understand the final paragraph devoted to the metabolic abnormalities reported in a variety of muscle diseases.

I. Informational content of a ^{31}P MR spectrum

Measurement of phosphorylated compounds concentrations in living cells is not easy. Traditional methods such as percutaneous needle biopsy and freeze clamping exhibit limitations especially related to possible partial degradation of phosphorylated metabolites during extraction. In addition, repeated measurements cannot be performed on the same muscle, making impossible the achievement of high-time resolution kinetics. Compared to analytical methods, ^{31}P MRS offers the opportunity of measuring non-invasively and continuously with a high time-resolution, the concentration of phosphorylated compounds involved in muscle energetics. Interestingly, direct biochemical and ^{31}P MRS measurements give comparable results [9-11].

A typical ^{31}P MR spectrum exhibits six to seven peaks corresponding to phosphocreatine (PCr), inorganic phosphate (P_i), the three phosphate groups of ATP (in position α , β et γ) and phosphomonoesters (PME) (figure 1). The $\text{P}\alpha$ signal of ATP displays occasionally an upfield shoulder corresponding to NAD^+/NADH . In between the PCr and P_i signals, the phosphodiester signal can be observed. This signal is usually assigned to glycerophosphorylcholine and glycerophosphoryethanolamine which can be detected as a small peak in normal muscle spectra (mainly from lower limb) and as a larger peak in patients with muscle dystrophy [12] illustrating membrane breakdown. Given the low sensitivity of the MR technique, the free metabolically active ADP concentration, which is only a tiny fraction of its total intracellular concentration, cannot be measured. It can however be calculated using the creatine kinase equilibrium where the total creatine content is taken as either 42.5 mM or considering that phosphocreatine represents 85% of the total creatine content [13]. Alternatively, [ADP] can be calculated by adjusting the CK equilibrium constant to the ionic conditions of the cell in order to avoid the errors that can arise at low pH and taking into account all the ionic species involved in the reaction catalyzed by the creatine kinase [13,14]. Similarly, AMP concentration can be calculated using the adenylate kinase equilibrium [13]. In the absence of biochemical data, ATP is often assumed to be normal and is used as the equivalent of an internal standard in order to calculate the concentrations of other metabolites.

Apart from the dynamic measurements of high-energy phosphate compounds, ^{31}P MRS offers the only non invasive way of assessing intracellular pH. Indeed, under conditions of physiological pH (one of the pK_a of P_i is 6.75) two forms of P_i coexist (H_2PO_4^- and HPO_4^{2-}). These two forms are exchanging so fast that only a single P_i signal is detected and the chemical shift (signal position on the horizontal axis) of this signal is a weighted average of both forms. Due to this sensitivity of the P_i chemical shift to pH, it is possible, with appropriate calibration curves, to measure muscle pH non invasively.

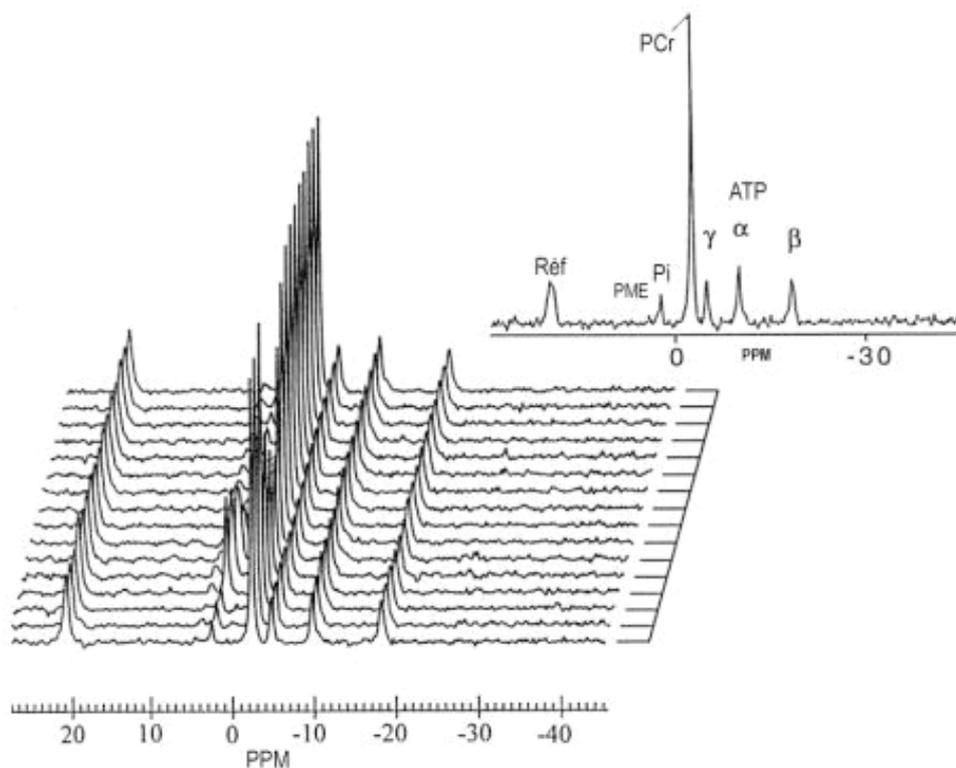
The quantitative measurements of high-energy phosphate compounds and pH allow to compute a number of derived metabolic variables such as, for instance, the oxidative phosphorylation potential and the free energy of ATP hydrolysis *in vivo*. Also, magnesium concentration can be calculated from the chemical shift of the ATP beta group [15-17].

Fat, fibrous tissue blood and extracellular fluid contribute no significant signal and mitochondrial metabolites are too tightly bound to interfere.

Figure 1

Typical series of ^{31}P MR spectra recorded in humans forearm flexor.

MR spectra have been recorded at 4.7T (Biospec 47/30 Bruker) during a standardized rest-exercise-recovery protocol with a time resolution of 15s. On the higher panel of the figure, is represented a single spectrum with the corresponding assignments. Ref: reference compound (phenyl phosphonic acid), PME (phosphomonoesters), Pi (inorganic phosphate), PDE (phosphodiesters), PCr (phosphocreatine), phosphate groups of ATP in position α , β and γ .



II. Technical considerations

The requirement for magnetic field homogeneity generally dictates that the muscle examined be positioned at magnet center and remain in a fixed position during data collection. In that respect, dedicated ergometers have been designed in each laboratory in order to investigate exercising muscles within superconducting magnets. So far, adductor pollicis [18], forearm and wrist flexor muscles [5,19,20], calf [21,22] and thigh muscles [23-26] have been investigated using ^{31}P MRS. MRS recordings have been sometimes coupled to other non invasive techniques such as electromyography [27,28] and gas exchange measurements [24]. Given the low magnetic resonance sensitivity of ^{31}P (6% of proton) and the low tissue concentrations of some of the relevant metabolites, MR signals are time-averaged over a period ranging from a few seconds to several minutes depending on the required signal to noise ratio and the desired time resolution. In addition, MR signal is detected with a surface coil over a relatively large muscle volume proportional to the surface coil radius making this signal a weighted average of the muscle fibers existing within the sampling volume. This has to be taken as a comparative item with histological and biochemical analyses which are often carried out on very small samples of tissues which may not give a representative picture of the biochemical state of the muscle. However, care has to be taken not to turn this advantage into a drawback while sampling exercising and non exercising muscles at the same time. This can be achieved when MRS is combined to MRI for i) proper localisation of the coil and ii) proper determination of the sensitive volume of the coil.

Yet only a small number of centers around the world utilize MRS routinely for investigations of metabolic changes surrounding muscle contraction. The labor intensive nature of this type of study and the necessity to combine efforts of scientists from different areas (biochemistry, physiology, physics, medicine) may be important in this regard in addition to the cost of the equipment. Studies involving hundreds of subjects have been rarely reported but in that respect MRS does not differ than other techniques. In terms of time, a typical rest-exercise recovery protocol almost requires an hour and several additional hours are necessary

for data processing. Initially manual, data processing has become automatic quite rapidly and recent analytical procedures performed on raw MR signals (time domain) rather on MR spectra (frequency domain) and incorporating prior knowledge have significantly improved the automatic analysis [29].

III. ATP homeostasis and ATP sources

One of the main contributions of ^{31}P MRS to muscle physiology is its ability to measure sequentially the variations of concentrations in PME, Pi, PCr, ATP as well as intracellular pH during muscle contraction and recovery. Biochemical reactions taking place in the myocyte are then directly reflected by specific perturbations on the spectrum (figure). Muscle energy production results in PCr and pH changes which in turn would modify ADP and Pi concentrations both of which ultimately stimulate ATP production. During contraction, the free energy stored in ATP (the ubiquitous substrate for energy production) is converted into mechanical energy via the interaction of two muscle proteins, actin and myosin. Muscle work is linked to hydrolysis of ATP. However, As illustrated in the figure, in normal subjects, the intracellular concentration of ATP remains unchanged during moderate exercise indicating an adequate balance between energy demand and supply [30]. A net ATP decrease has been only reported when exercise intensity was by far larger [31] and interestingly such a net ATP consumption has rarely been reported in patients with metabolic disorders [32-34]. The balance between ATP demand and ATP supply is ensured by the three main sources of ATP within the muscle and each of them can be accurately quantified from the PCr and pH time-dependent changes in exercising muscle and throughout the recovery period.

III.A. PCr changes in exercising muscle

PCr is largely recognized as an intracellular buffer (some authors have also reported PCr as an energy shuttle between production and consumption sites) allowing to compensate for any shortfall in either non oxidative or oxidative ATP production. Across the rest to work transition, [ATP] in working muscle is maintained by phosphocreatine breakdown in a reaction catalysed by creatine kinase. After a few seconds (if not immediately) both glycolysis and oxidative phosphorylation are activated [35,36]. The time-dependent changes in PCr has been described as either mono or biexponential likely in relation to exercise intensity [37]. From these changes, the involvement of PCr breakdown in energy production can be easily calculated as a decreasing contribution. In addition, considering PCr as the major energy source at the onset of exercise [38], the initial rate of PCr consumption can be calculated and it can be used to infer the energy cost of contraction when power output (W) is taken into account.

III.B. pH changes in exercising muscle, buffering components and glycolytic ATP production

On initiation of muscle contraction or at times when the workload or rate is increased, glycogen breakdown is rapidly activated to provide substrate for glycolysis. Pyruvate that is not used as a substrate by the mitochondria accumulates and the combined production of lactate and ATP hydrolysis lowers pH [39,40]. The extent of the change in pH is a balance among lactic acid production proton efflux, ATP hydrolysis and the pH raising effect of PCr breakdown. Interestingly, all these components could be quantitatively analysed using ^{31}P MRS.

With a high enough time-resolution such that an initial alkalosis (dpH/dt) can be measured at the onset of exercise, muscle buffering capacity can be calculated as the ratio of the expected pH changes (due to PCr breakdown) and the measured pH change. This composite buffering capacity includes bicarbonate and non bicarbonate compounds. The contribution (β_x) of inorganic phosphate (Pi) and sugar-phosphate (PME) to this composite buffering capacity can be determined using the standard formula of a buffer dissociation [41]. Exercising muscle is usually assumed as a "closed" system [41-43] and buffer capacity due to bicarbonate is set to zero.

These values are used for the determination of the glycolytic rate of ATP production which can be calculated differently according to the type of exercise. Under ischemic conditions, given that ATP supply is mainly supported by PCr breakdown and glycolytic ATP production, the glycogenolytic flux can be simply calculated as the difference between the total rate of ATP turnover and the rate of ATP production from PCr breakdown at any time of exercise. When ATP demand is supported by both non oxidative and aerobic sources (mixed exercise), the calculation of the glycolytic rate takes into account each component of the H^+

balance, i.e., overall H^+ production associated with nonoxidative ATP production, H^+ efflux, and H^+ uptake associated with PCr breakdown [33,44].

As described in [41], the rate of H^+ efflux cannot be directly estimated during exercise but has to be analysed from the coupled analysis of pH changes (usually an additional acidosis) and PCr resynthesis during the initial part of the recovery period following exercise. Indeed, during the post-exercise recovery period, pH recovers back towards its resting value despite a continuous proton load from PCr resynthesis :

III.C. PME changes in exercising muscle, glycogenolytic and glycolytic ATP productions

The PME signal in ^{31}P MR spectra comes from hexose phosphates i.e. glycolytic intermediates such as glucose 6-phosphate (G6-P) and fructose 6-phosphate (F6-P). Alternatively IMP and AMP could contribute to the PME signal but only when ATP homeostasis is compromised and a net ATP degradation is measured. An accumulation of PME occurs normally during exercise likely as an imbalance between glycogenolytic and glycolytic fluxes [13,45]. Indeed, glycogenolytic flux often exceeds glycolysis flux [46] and glycogenolysis supplies glycolysis with most of its substrate G6-P. Glycogenolysis flux could then be quantified from the accumulation of PME and taking into account the glycolytic flux [13,47].

The analysis of PME accumulation during exercise has delineated whether glycolysis is activated or not as a mass-action response to glycogenolysis. Actually, PME accumulation occurs before the significant activation of glycolysis [13,48] indicating that the glycogenolysis production of PME is not sufficient to trigger glycolysis flux and that the two pathways differ in their sensitivities to the signals that control flux i.e. metabolites such as P_i and ADP and calcium. Instead, the glycolytic pathway is controlled at one or more sites downstream of glycogenolysis [49].

Pathological changes in PME encompass lower and higher accumulation of PME occurring in proximal and distal glycogenoses respectively [30,50]. In the former situation, PME does not accumulate as a result of impaired glycogenolysis whereas in the latter situation a reduced PFK activity (or another enzyme downstream of the glycolytic pathway) enlarges the already existing imbalance between glycogenolysis and glycolysis and accounts for the larger accumulation of PME. Together with these abnormal changes, exercise-induced acidosis is either limited [50] or does not exist [30] again as a result of the reduced activities of either glycogenolysis and/or glycolysis [30,50].

III.D. PCr and pH changes in muscle after exercise, aerobic ATP production

During the post-exercise period, PCr and pH both recover back to their respective value with a lag time for pH. As previously underlined, this lag time accounts for the proton load from PCr resynthesis and this can be used in order to calculate proton efflux [51] which is modulated by blood flow and activities of various transporters. The PCr recovery kinetics provides information regarding oxidative ATP production. Indeed, it has been clearly demonstrated that PCr resynthesis in recovery is purely aerobic [52,53], one of the illustrations being the absence of PCr resynthesis during the post-stimulation ischemic period whereas all the potential controllers of glycolysis i.e. P_i and ADP were by far larger than their respective K_m [53]. In addition, the rate of PCr recovery is slowed down in mitochondrial disorders [54-56] and cardiac failure [57,58] as a result of impaired mitochondrial respiration, improved in athletes [7,59-61] consistent with an increased mitochondria content and/or activities of enzymes involved aerobic ATP production. Also, it has been shown that PCr recovery kinetics can be accelerated with increased oxygen supply [62,63] (at least in exercise-trained subjects) and following anti-asthenic treatment [56]. The independence of recovery data with respect to stimulation frequency and exercise intensity [52,64] is also of importance for the utilisation of recovery data as indices of aerobic capacity. Comparative analyses of *in vivo* MR data and *in vitro* measurements of mitochondrial enzymes activities have shown good agreements [65-67]. Overall, PCr recovery data are considered as robust measures of mitochondrial function as long as the dependence with end-of-exercise parameters such as intracellular acidosis is properly taken into account [19,60,68]. The PCr recovery kinetics is usually considered as a monoexponential function with PCr increasing from the end-of-exercise value to the resting value with a kinetic constant k .

IV. Pathological changes assessed with ^{31}P MRS

Given its ability to follow high-energy phosphate compounds and pH changes during transitions from rest to exercise and exercise to rest, ^{31}P MRS has a clear potential for delineating the metabolic abnormalities of a particular myopathy thereby providing unique diagnostic indices. Skeletal muscle makes up about 40% of body mass. Its metabolism largely influences whole-body homeostasis and disorders in other organ systems should have an impact on muscle energetics. ^{31}P MRS have provided key information for primary metabolic disorders affecting muscle energy production (table1) but also for secondary problems of muscle metabolism associated with common conditions such as heart failure, renal failure and thyroid disease.

As illustrated by the results in table 1, the specificity of MRS data does not reside in any single measurement but in the pattern of abnormalities that distinguish particular types of primary or secondary muscle diseases. For instance, the PCr recovery rate measured after exercise is slow in a large variety of disorders indicating that in all of them oxidative ATP production is impaired (given that, as illustrated in paragraph III.D, PCr kinetics throughout the recovery period is exclusively oxidative). However, with ^{31}P MRS data one could go further than that and determine the causative factors of this impaired aerobic energy production. The lack of acidosis in Mc Ardle disease [30] indicates that impaired glycogenolysis and then lack of substrate is the causative factor of impaired oxidative ATP production. On the contrary, in mitochondrial myopathy, the combined analysis of PCr and pH time-dependent changes point towards a mitochondrial deficiency as accounting for the impaired muscle energetics [32,55,69]. A final example of the specificity of MR results is related to the impaired aerobic energy production in idiopathic inflammatory myopathy [70]. In that particular case, the reduced rate of PCr recovery is related to an abnormal rate of pH recovery illustrating that impaired blood flow might be responsible for the abnormal aerobic production as a result of the muscle inflammatory process [70].

Deficits in ATP production caused directly by gene defects in skeletal muscle or indirectly by hormonal changes, virus infection, inflammatory process, renal failure, ischemic disease, respiratory disease..... could alter the normal MRS pattern thereby providing one or more diagnostic indices. However one should be cautious with the interpretation of the results. For instance, two brothers with genetic defects of MELAS showed similar reduction in maximum aerobic capacity while one was with 85% mutated DNA and the other with only 4% mutated DNA [71]. This result could be interpreted as a discrepancy between *in vivo* and *in vitro* measurements or as indicative of the absence of a clear threshold for the A3243G mutation in skeletal muscle. These results have important implications for the understanding of the phenotypic expression of mt DNA disease.

An additional step into the understanding of ^{31}P MR metabolic indices resides in the quantitative analysis initially developed by Kemp *et al.* [72]. With this type of analysis, one can further distinguish between reduced muscle mass and/or reduced muscle efficiency and reduced oxidative capacity as accounting factors of abnormal exercise-induced metabolic changes. Indeed, for a given muscle mass and a given mechanical power output, the initial rate of ATP synthesis is by definition inversely proportional to metabolic efficiency [73]. As illustrated in previous paragraphs, both aerobic and non oxidative contributions to energy production can be calculated from the analysis of PCr, Pi, Pme and pH dynamics. These changes depend on mechanical work, muscle mass and metabolic efficiency (the latter two variables are combined as effective muscle mass) [33]. Generally speaking, greater PCr and pH changes should be recorded if the ratio of work to effective muscle mass is increased or if aerobic ATP synthesis is decreased. Such a comparison has been performed in a study of dialyzed uremic patients [33]. Raw data recorded in this group of subject illustrated a larger PCr consumption while exercise duration and the corresponding power output were reduced. pH time-dependent changes were normal and the analysis of the recovery period disclosed a 50% reduction of the maximum aerobic capacity. From the initial increase in the PCr consumption rate, one can infer a reduced effective muscle mass. Given that muscle mass (measured separately) was similar in both groups of subjects, reduced metabolic efficiency can be suspected as accounting for the greater exercise-induced metabolic changes. In addition, considering that both oxidative and glycolytic contributions to energy production were increased in absolute terms but normal when energy cost was taken into account, it can be proposed that reduced muscle efficiency rather than impaired aerobic ATP production likely accounts for the greater PCr changes in exercise [33].

Table 1 : Pathological changes measured using ^{31}P MRS (rest-exercise-recovery protocol)

<i>Pathological situation</i>	<i>Rest</i>	<i>ATP depletion</i>	<i>PCr depletion</i>	<i>Intracellular acidosis</i>	<i>Rate of PCr recovery</i>	<i>Ref</i>
Mitochondrial Myopathy	Reduced PCr/Pi	No	Larger	Limited	Slow together with faster rate of pH recovery	[32,55,69]
McArdle disease	Increased PCr/ATP	No	Larger	Limited	Slow	[30,80]
PFK deficiency	Increased PCr/ATP	Yes	Larger	Limited	Slow	[81,82]
Dystrophy (Duchenne and Becker)	Increased pH and PDE	no	Larger	Normal	Normal	[83,84]
Myotonic dystrophy	Decreased phosphorylation potential	No	Larger	Limited	slow	[85,86]
sIBM	Increased pH Increased Pi/ATP	Normal	Normal	Normal	Normal	[87,88]
Malignant Hyperthermia	Increased PDE	No	Larger	Excessive	Normal	[89,90]
IIM (Poly and dermatomyositis)	Low PCr/ATP and Pi/ATP	No	larger	Normal	Slow together with rate of pH recovery	[70,91]
Renal failure	Increased Pi/ATP	No	larger	Normal	Slow	[33,92]
Chronic respiratory failure		No	Larger	Excessive	Slow	[93-95]
Congestive Heart failure		No	larger	Excessive	Slow	[96,97]
Peripheral vascular disease		No	Larger	Excessive	Slow together with rate of pH recovery	[13,98,99]
Thyroid disease (Hypo)		No	Larger	Smaller	Normal together with slow rate of pH recovery	[100]
Thyroid disease (Hyper)				Excessive	Normal	[101]
Essential hypertension				Smaller	Normal together with faster rate of pH recovery	[102]

V. Future investigations

Employing non-MR based techniques simultaneously with MRS can aid in data interpretation and will certainly broaden the scope of muscle investigation. Near infrared spectroscopy (NIRS) provides data on state of tissue oxygenation but reliability has still to be proven mainly on the basis of comparative and/or combined analyses using MRS and NIRS [74]. Electromyography is another technique of interest which can be used to study correlation between metabolic and electrical changes and provide interesting features regarding muscle fatigue [75]. Combined with MRS, MR imaging techniques can also help understanding muscle energetics. For instance, MRS data showing altered metabolites concentrations need to be interpreted with caution. Indeed, metabolites concentrations may be altered but the presence of fatty or fibrous infiltration can introduce false-positive results in a sense that data might not represent concentrations in the actual muscle fibers. Functional MRI can also be of interest for understanding muscle activation during exercise. Based on T2 changes due to uptake or redistribution of fluid within the exercising muscle,

functional MRI is considered as a semi-quantitative method of assessing muscle recruitment during exercise [76]. For instance, in Mc Ardle's disease, abnormal glycogenolysis is coupled to abnormal exercise-induced T2 changes [77,78]. Similarly, subnormal exercise-induced T2 changes increase can be recorded in other metabolic myopathies [79]. However, muscle functional MRI is still underutilized although its used is certainly well suited in the context of muscle energetics and for documenting muscle activation pattern in pathological conditions and in subjects with very highly trained to exercise.

VI. References

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