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31-P NMR CHARACTERIZATION OF THE METABOLIC ANOMALIES ASSOCIATED WITH THE LACK OF GLYCOGEN PHOSPHORYLASE ACTIVITY IN HUMAN FOREARM MUSCLE [1]

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Exercise-induced changes in phosphorus-containing metabolites and intracellular pH (pHi) have been studied in the finger flexor muscles of 3 patients with glycogen phosphorylase deficiency (McArdle's disease) in comparison to 14 healthy volunteers. At rest, no difference was observed for PCr/Pi ratio and pHi while patients exhibited a higher PCr/ATP ratio (5.91 ± 0.98 vs 4.02 ± 0.6). At end-of-exercise, PCr/Pi was abnormally low (0.51 ± 0.19 vs 1.64 ± 0.37) whereas no acidosis was observed. The slow recovery of PCr/Pi ratio indicates an impairment of oxidative capacity accompanying the defect in the glycogenolytic pathway. The failure to observe a transient Pi disappearance at the onset of recovery (an index of glycogen phosphorylase activity) can be used in conjunction with the lack of exercise acidosis as a diagnostic index of McArdle's disease.

McArdle's disease, an inborn error of metabolism caused by a lack of glycogen phosphorylase activity in skeletal muscle was first described in 1951 (1). Characteristic features of McArdle's disease are exercise-induced muscle pain, cramping and premature fatigue. On open-muscle biopsy, direct enzyme assay shows decrease glycogen phosphorylase activity and histochemical analysis demonstrates excess glycogen in muscle fibres (2,3). The lack of lactic acid increase in the venous blood following handgrip exercise performed under ischemic conditions has been proposed as a diagnostic index (4,5). However, it has been shown that this test may not detect patients with partial expression of McArdle's disease (6).

31-P NMR spectroscopy, now widely introduced into clinical investigations, allows to follow metabolic events surrounding muscular exercise in a non invasive manner 8-11). The first 31-P NMR examination of a case of suspected McArdle's disease has been reported by Ross *et al.* in 1981 (12). The lack of acidosis during exercise is the main criterion on which diagnosis was made based on the observation that the chemical shift of Pi peak was not altered. Since then,

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31-P NMR spectroscopy has been used to document the metabolic basis of this abnormally rapid fatigue during strenuous exercise (13-16) as well as to investigate other muscular disorders (17). It has also been used as an additional diagnostic tool or as a way to determine the metabolic effects of various therapies (18-20). However, other metabolic myopathies involving deficiencies in more distant enzymes of the glycogenolytic pathway give similar 31-P magnetic resonance patterns with no (or very limited) acidosis upon muscular effort. Also, it has been reported that heterozygous subjects with glycogen phosphorylase deficiency displayed significant acidosis during exercise performed under aerobic conditions (13). We have recently reported a 31-P NMR study at 4.7T of the metabolic response to muscular exercise in control subjects, focusing on the time-dependent variation of prephosphofructokinase metabolites in relation to inorganic phosphate (Pi) level (10). It was then interesting to investigate the metabolic events surrounding muscular exercise in 3 patients with glycogen phosphorylase deficiency who were unable to utilize their glycogen store.

Material and Methods

Subjects: The study was conducted on the dominant forearm of three patients with McArdle's disease (aged 35 - 56 yr) and of fourteen healthy subjects (aged 20 - 45 yr). Each patient had lifelong exercise intolerance and cramps. Histochemical and biochemical analysis demonstrated a lack of myophosphorylase for the three patients. All subjects participated after providing informed consent.

NMR Spectroscopy: NMR spectra were recorded at 4.7 T on a Bruker 47/30 Biospec system equipped with a horizontal superconducting magnet (bore diameter:30 cm) operating at 81.15 and 200.14 MHz for 31-P and 1-H respectively. The subjects sat on a chair by the magnet and inserted their arm horizontally into the magnet bore. The radiofrequency coil used to collect NMR data was a 50 mm-diameter double-tuned surface-coil. It was positioned over the *flexor digitorum superficialis* muscle. The forearm was placed approximately at the same height as the shoulder to ensure a good venous return. Optimization of the field homogeneity was done by monitoring the 200.14 MHz signal from the muscle water and fat protons. NMR data were acquired in 1 min blocks with the use of a pulse repetition rate of 32 per min, a radio frequency pulse duration of 55 μs and were collected into 4K data files. Spectra were time-averaged over 1 min. and sequentially recorded during 3 min. of rest, 3 min. of exercise and 20 min. of recovery. A 15 Hz line broadening function was applied before Fourier transformation. A micropipette filled with a solution of methylenediphosphonate (MDP) was positioned at the surface coil center to accurately monitor global changes in spectral intensity.

Exercise protocol: After 3 min of rest, exercise consisted of finger flexions at 1.5 s intervals for 3 min lifting a 6 kg weight. The patient's arm was restrained throughout the protocol which included 20 min of post-exercise recovery. All 3 patients were able to perform the exercise during 3 min.

Data analysis: Relative concentrations of metabolites were determined by integration of the respective resonances. In order to best compensate for differential saturation effects, T1 relaxation times at 80.15 MHz were measured on resting human forearm muscle by the saturation-recovery method for PCr (4.31 ± 0.94 s), Pi (4.54 ± 0.95 s) and βATP (2.41 ± 0.45 s). Due to the inhomogenous radiofrequency field generated by a surface coil, saturation factors were estimated by comparing partially saturated spectra recorded with a 2 s interpulse delay with fully relaxed spectra. Saturation factors were 1.71 for PCr, 1.87 for Pi, 1.27 for ATP and 1.63 for Pme. Intracellular pH was calculated from the chemical shift of Pi relative to PCr at -2.45 ppm with respect to 85% H_3PO_4 (21).

Statistics
Results are presented as mean ± sd. Statistical significance of differences between each group was determined by unpaired, two tailed t tests with $p < 0.01$ chosen to be significant.

Results and Discussion

Figure 1a displays the time-dependent changes in Pi relative concentration during rest, exercise and recovery for control subjects (CS) and Mc Ardle's patients (mAP). As we previously reported (9,11), CS display a wide range of metabolic responses for PCr breakdown, Pi accumulation and pH decrease during exercise. We have shown that PCr/Pi ratio calculated at the end of exercise is linearily correlated to end-of-exercise acidosis thereby allowing standardization of the experimental protocol without recourse to MVC measurements (11). Also, we have established linear relationships linking the kinetics of recovery for PCr and PCr/Pi ratio to the intracellular pH reached at the end of exercise (9). Since in CS exercise always results in pH decrease, there were no CS matching the pH variation recorded for mAP which would have allowed us the use of those invariant relationships. Therefore, we chose to compare the mean muscular response to exercise recorded for CS to the metabolic response recorded for mAP keeping in mind that intracellular acidosis recorded during exercise determines the value of PCr/Pi ratio measured at the end of exercise and the kinetics of PCr/Pi recovery.

PCr/Pi ratio and pH values averaged on the three spectra recorded at rest show no significant differences between mAP and CS. Nevertheless, PCr/ATP ratio was significantly higher in mAP(Table 1).This result is in agreement with a previous study (22) and can be accounted for

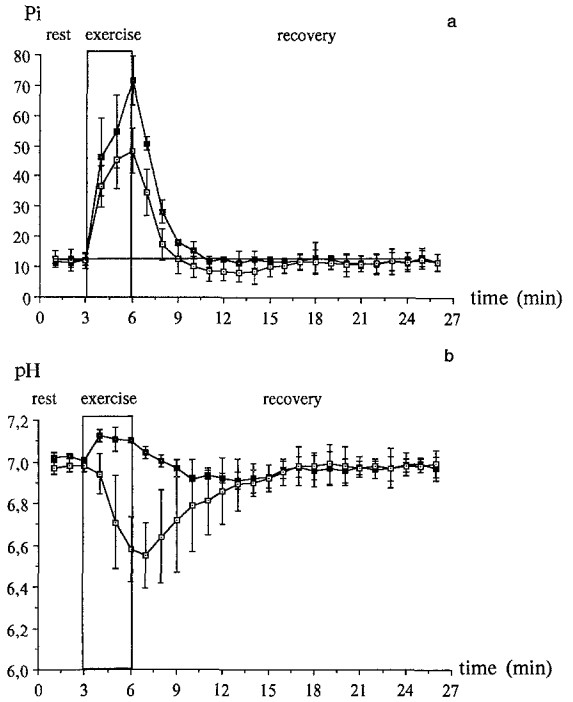

Figure1. Time-dependent changes in Pi(a) and pH content (b) during the experimental protocol in control subjects (open squares) and McArdle's patients (closed squares). Results are presented as mean ± sd.

Table1. Measurements of metabolic indices recorded during the rest-exercise-recovery protocol

		Control Subjects (n=14)	Patients (n=3)	p< 0.01
REST	pH	7.00 ± 0.02	7.01 ± 0.01	ns
	PCr/Pi	8.88 ± 0.91	7.47 ± 1.03	ns
	PCr/ATP	4.02 ± 0.60	5.91 ± 0.98	*
EXERCISE	pH	6.58 ± 0.16	7.08 ± 0.03	*
	PCr/Pi	1.64 ± 0.37	0.51 ± 0.19	*
	PCr	50.97 ± 11	30 ± 3.46	*
	Pme/Pme_r	4.04 ± 0.75	not detected	*
RECOVERY	d(PCr/Pi)/dt	3.20 ± 1.07	1.30 ± 0.26	*

Pme_r indicates the Pme level at rest.
d(PCr/Pi)/dt is expressed in PCr/Pi units/min.

by a reduction in intracellular ATP level in patients with myophosphorylase deficiency. During exercise, excessive PCr breakdown correlated to an abnormally high Pi accumulation was noted as determined by the very low value of PCr/Pi ratio (table 1). In mAP, we failed to observe a phosphomonoester (Pme) resonance at rest and no increase in Pme level was noted during exercise, as it was the case in control subjects (table 1). This result confirms our previous observation that the Pme peak is mainly due to sugar phosphates produced by glycogenolysis and not to IMP although it has been reported that in Mc Ardle's disease, IMP and ammonia production largely exceed normal values (23). This abnormally high production has been attributed to an activation of the myokinase/myoadenylate deaminase pathway. We have previously reported (10) that in CS, exercise was correlated to a 3 to 5-fold increase in Pme (mostly glucose 6-phosphate). It has been shown that during short-term intense exercise, glycogen was the major fuel used and that prephosphofructokinase metabolites accumulation could indicate the initial imbalance among the phosphorylase and phosphofructokinase activities (24). Glycogen phosphorylase in muscle is responsible for glycogen degradation, using inorganic phosphate to produce glucose 1-phosphate which in turn enters the glycolytic

pathway. Therefore, abnormally high Pi accumulation in mAP has to be linked not only to excessive PCr breakdown but also to inefficient glycogen phosphorylase which prevents Pi incorporation into the Pme pool.

As observed on fig. 1a, transient Pi disappearance observed in CS during the initial period of recovery was not observed in mAP. This transient Pi disappearance observed in CS during the initial recovery period is related to Pi incorporation into the Pme pool during exercise and to slow post-exercise Pme recovery (10). Failure to observe both Pme accumulation during exercise and transient Pi disappearance during recovery could be then considered as indices of glycogen phosphorylase inactivity in glycogen deficient muscles. Accumulation of large amounts of Pi produced in skeletal muscle could inhibit myofibrillar calcium and sodium-potassium ATPases reactions and then could account for the premature muscle fatigue and cramping of mAP. (25).

Fig. 1b displays the time-dependent evolution of intracellular pH recorded during the rest-exercise-recovery protocol. CS displayed a wide range of exercise-induced acidosis whereas, as observed earlier (12-15), we failed to observe any decrease of intracellular pH during exercise in mAP. On the contrary, we noted a pH increase from 7.01 ± 0.01 to 7.08 ± 0.03 which could be accounted for by H^+ consumption during active PCr breakdown. Failure to observe any decrease in pH during exercise has to be linked to the unability of mAP to utilize glycogen as a source of energy, thus preventing lactic acidosis.

As indicated on table 1, the kinetics of recovery of PCr/Pi ratio were abnormally slow in mAP compared to control subjects suggesting a possible association of an oxidative disorder to the blockade of the glycogenolytic pathway. It has been previously shown that oxygen uptake was reduced in mAP down to 35 to 50% of normal (26,27). A reduced rate of formation of mitochondrial reducing equivalents would ultimately limit the rate of oxidative phosphorylation and oxygen consumption. Also, it has been reported that inefficient anaerobic glycogenolysis could impair muscle oxidative metabolism by reducing the production of pyruvate, which is the preferred substrate of oxidative metabolism during heavy exercise (25). Patients with myophosphorylase deficiency can use branched chain amino acids (BCAA) (28) as an alternative energetic supply thereby producing a large amount of ammonia. Some authors reported that excessive metabolism of BCAA could lead to a reduction of intermediates in the citric acid cycle which in turn could impede aerobic oxidation (28)

In conclusion, these findings are consistent with observations that the unavailability of glycogen as an oxidizable fuel resulting from glycogen depletion in control subjects (29) or from enzyme deficiency in patients devoid of muscle glycogenolysis, limits oxidative capacity. The finding of abnormally low kinetics of recovery for the PCr/Pi ratio is consistent with the hypothesis that muscle oxidative capacity is impaired in McArdle's patients because the metabolic block at the glycogen phosphorylase level limits the availability of substrates for maximal oxidative phosphorylation. Finally, we observed in agreement with others that Pi accumulation was significantly more important in mAP. This finding correlated to the absence of Pme changes during exercise and the failure to observe transient Pi disappearance during the initial period of recovery confirms our previous interpretation of the changes in NMR spectra during post-exercise recovery (10). It provides additional metabolic indices for diagnosis of glycogen phosphorylase deficiency.

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