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Pi trapping in glycogenolytic pathway can explain transient Pi disappearance during recovery from muscular exercise

A ^{31}P NRM study in the human*

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^{31}P NMR spectroscopy at 4.7 T has been used to follow changes in phosphorylated metabolites and pHi in the flexor digitorum superficialis muscle of 15 healthy volunteers subjected to a rest-exercise-recovery protocol. Phosphomonoesters (Pme) increased during exercise and exhibited a delayed recovery to resting level. During early recovery, Pi fell below resting concentration without correlated PCr oversynthesis while Pme level stayed above its resting value. The sum Pi + Pme remained constant. These observations suggest that Pi could be trapped into the glycogenolytic pathway during exercise leading to Pme production. This trapping and the slow Pme recovery could account for transient Pi disappearance observed during recovery.

In vivo NMR spectroscopy; Phosphorylated metabolite; Inorganic phosphate; Phosphomonoester; Human muscle

1. INTRODUCTION

Since the first study by Hoult et al. [1] in rat muscle, ^{31}P NMR spectroscopy has been extensively used to investigate metabolic processes related to muscular exercise and recovery. Using exhausing and non-exhausting aerobic dynamic exercise, studies have focused on the link between ATP hydrolysis by actomyosin-ATPase and ATP resynthesis [2-4] and a better understanding of muscle energy regulation has been reached. In humans, ^{31}P NMR spectroscopy provides now a new tool for medical diagnosis based on the comparative analysis of the response to muscular work between normal subjects and patients [5-7].

^{32}P NMR spectroscopy allows to monitor changes in phosphorylated tissue metabolites during muscular exercise and recovery, as compared to rest. Intracellular pH can also be measured [21]. An interesting observation has been the disappearance of the Pi signal during early recovery following exercise without correlated PCr oversynthesis [4-9]. This effect has been so far accounted for on the basis of a Pi redistribution among cell compartments and Pi has been proposed to enter transiently an NMR invisible pool [4-9]. We report here a ^{31}P NMR study of human muscle, at 4.7 T, which focuses on Pi disappearance and the possible relationship with other phosphorylated metabolites, mainly phosphomonoesters (Pme).

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2. EXPERIMENTAL

2.1. *Subjects* The study was conducted on the dominant forearm of 15 informed healthy volunteers. All of them were right-handed. They were not involved in any arm training, had no physical limitation to exercise and were between 22 and 35 years of age.

2.2. *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. A 50 mm double-tuned surface-coil 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. Optimisation of the field homogeneity was done by monitoring the 200.14 MHz signal from the muscle water and fat protons. Data were acquired following 55 μs radiofrequency pulses applied at 2 s intervals. Spectra were time-averaged over 1 min (32 scans) 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.

2.3. *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 volunteer's arm was restrained throughout the protocol which included 20 min of post-exercise recovery.

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2.4. *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 [8].

3. RESULTS

Phosphomonoesters (Pme) resonate as a broad signal centered at 4.5 ppm. They consist mostly of glucose 6-phosphate [10,13]. At rest, Pi and Pme have been accurately measured thanks to the increased resolution obtained at 4.7 T. For all subjects PCr/Pi and PCr/ATP ratios were respectively 9.7 ± 0.7 and 3.00 ± 0.45 in agreement with previous studies [3-4]. Intracellular pH was 7 ± 0.02. The resting Pme content represented between 2.8% and 5% of the PCr resting content.

During the 3 min of exercise, PCr decreased as a mirror image of the Pi increase while intracellular acidosis was observed. Typical profiles are shown in Fig. 1. In some cases the width and shape of the Pi signal were altered, leading eventually to two distinct resonances. Concomitantly the Pme signal increased, appearing as a broad resonance and never exceeding the Pi peak. The ATP level was maintained at its resting value (Fig. 1C).

At the completion of the exercise period, the extent of PCr breakdown, the PCr/Pi ratio and intracellular acidosis differed among subjects. Intracellular pH ranged from 6.33 to 6.78 and PCr/Pi ratio varied between 1.1 and 1.8. As the exercise was stopped, Pme level was maximum and represented between a 3- and a 5-fold increase relative to the resting value (Fig. 2A). This rise was independent of intracellular acidosis.

The kinetic profiles of recovery (Figs 1 and 2) indicate a rapid return of Pi level to its resting value. Recovery of PCr was slower and well described by a single exponential curve for all subjects (Fig. 1A). Interestingly, after 3 min of recovery the Pi signal was reduced to a level below its pre-exercise value while PCr recovery was 80% (Fig. 1B). At this time, the Pme signal was still higher than its resting value and remained so for an additional 6 min (Fig. 2A). Pi reappearance after 9 min of recovery concurred with Pme return to its resting value. Resting pHi was recovered in about 5 min after a transient initial decrease during the first min of recovery due to PCr resynthesis [4]. Throughout the whole protocol ATP homeostasis was maintained as well as the total phosphorus content (Figs 1C and 2C). The reference MDP signal was stable (Fig. 2D).

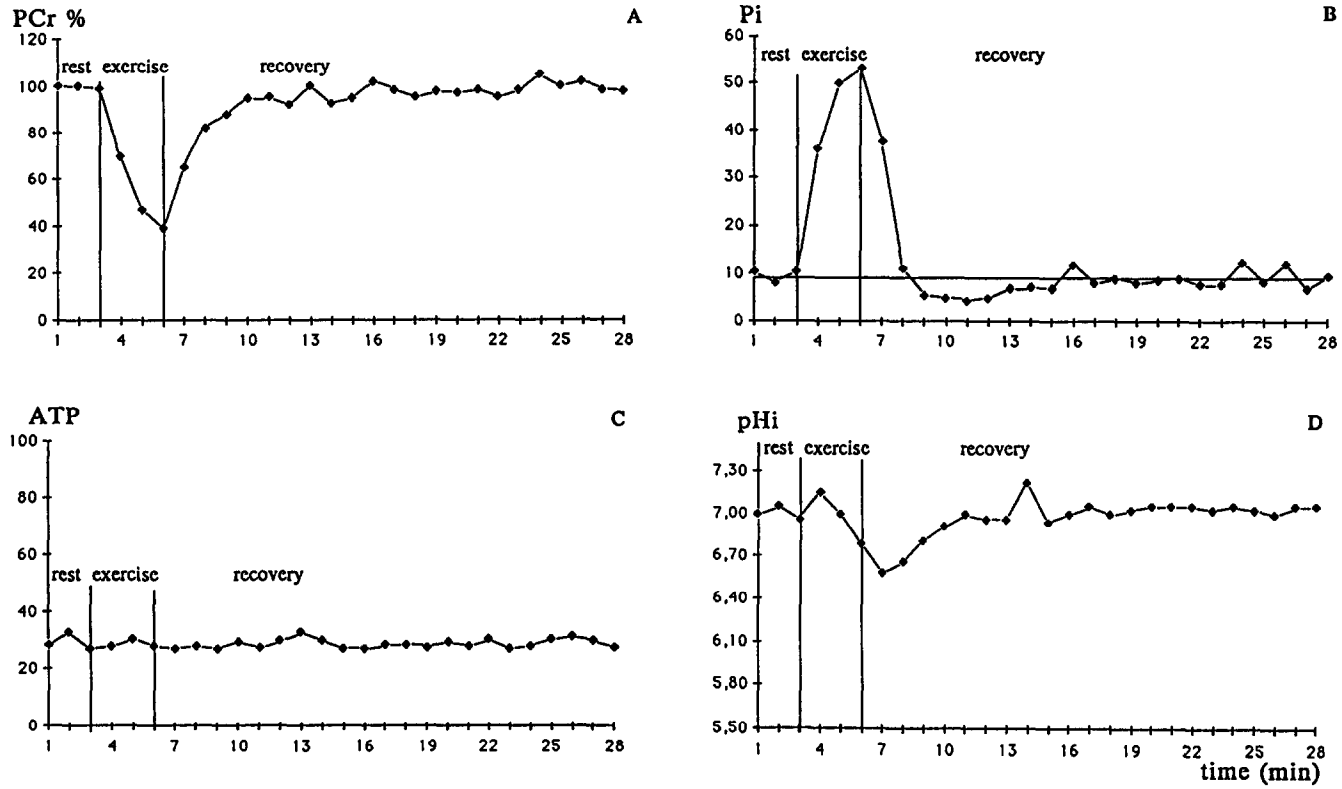

Fig. 1. Typical time-dependent changes in PCr (A), Pi (B), ATP (C) concentrations and intracellular pH, pHi (D) during rest, exercise and recovery. All values are expressed relative to the resting PCr concentration taken as 100.

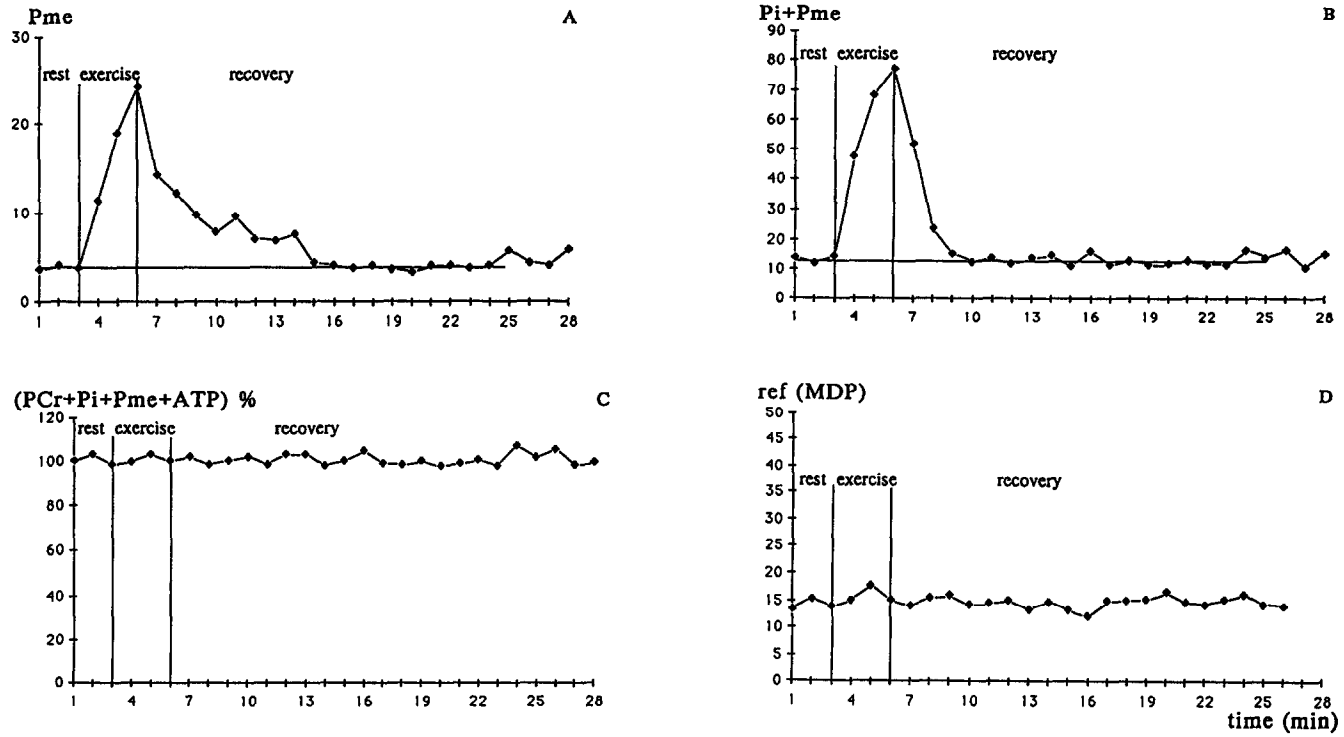

Fig. 2. Typical time-dependent changes in Pme (A), Pi + Pme (B), PCr + Pi + Pme + ATP (C), and the reference compound, ref (MDP) (D) during rest, exercise, and recovery. All values are expressed relative to the resting PCr concentration except the sum PCr + Pi + Pme + ATP which is referred to its own resting value.

4. DISCUSSION

We have recorded a 3- to 5-fold increase in Pme (mostly glucose 6-phosphate) during exercise. This observation agrees with previous studies by Chasiotis et al. [12] who reported in humans an exercise-induced formation of fructose 6-phosphate, glucose 6-phosphate and glucose l-phosphate representing respectively a 4-, 5- and 2-fold increase. Other human and animal studies have also shown the generation of Pme during exercise [10,13,14]. Our results suggest firstly, that anaerobic glycolytic activity leading to lactate and H^+ production, is concurrent to both PCr hydrolysis and Pi production and secondly that Pi could be an activator of glycogenolysis leading to Pme production. Thus the Pme increase could be accounted for by Pi trapping into the glycogenolytic pathway via glycogen phosphorylase. In various enzyme deficiencies involving glycogenolysis and glycolysis, Jehenson et al. have observed Pme accumulation during exercise particularly in phosphofructokinase and phosphoglyceratekinase deficiencies [6]. They reported that in such cases Pi increased much less than in control subjects during exercise, while PCr decreased. In contrast Pme increased substantially further indicating that Pme formation is correlated to Pi production during exercise. Pme production probably accounts for rate limiting steps in the glycogenolytic pathway switched on at the onset of exercise in the normal subjects. As suggested recently by Ren et al. [16] this accumulation of Pme (prephosphofructokinase located metabolites) during exercise could indicate the initial imbalance in the phosphorylase and phosphofructokinase activities.

During the post-exercise Pi undershoot, we did not observe a related overshoot of PCr. It is noteworthy that the sum of Pi and Pme concentrations during the Pi undershoot equalled the sum of Pi and Pme at rest (Fig. 2B). In addition, the kinetic profiles of Pi and Pme recovery following exercise were identical (Figs 1B and 2A). These observations clearly indicate an exchange between Pi and Pme. We can then assume that Pi redistribution in the Pme pool during exercise and slow Pme recovery can account for Pi undershoot during recovery. Recently Chaliss et al. [15], using gated ^{31}P NMR spectroscopy in rat skeletal muscle reported that a temporary decrease in NMR visible (PCr + Pi) could be explained by the large difference between the rate of Pi disappearance and PCr resynthesis. We also noted that the sum (PCr + Pi) transiently decreased under the resting value but we observed concomitantly that the sum (PCr + Pi + Pme) was the same as the resting value further indicating a direct exchange between Pi and Pme both during exercise and recovery. Interestingly investigations of energetic metabolism during exercise of a patient who complained of muscle pain have clearly indicated that Pme were used and not

produced during exercise and that during recovery Pi level always exceeded the resting value (unpublished results). Finally, examination of a patient with McArdle's syndrome showed that there was no disappearance of Pi during early recovery from exercise suggesting that the transient Pi disappearance observed in control subjects was directly linked to phosphorylase activity.

Response to muscular exercise consists initially of a PCr breakdown and a rise of Pi. We can now propose that part of the cytosolic Pi pool would then be trapped into the glycogenolytic pathway leading to Pme production and partial Pi depletion. On recovery, depletion of remaining cytosolic Pi by transfer into the mitochondrial compartment is related to PCr resynthesis and Pi temporarily disappears because of the still high Pme level. As Pme concentration returns to its resting value, Pi reappears. At last, Pi and Pme recover simultaneously to their resting values. Unequivocal assignment of all compounds contributing to the irregular hump in the Pme region is still necessary to fully describe the activation mechanisms of glycogenolysis during both exercise and recovery. However, our experiments clearly show that Pi undershoot during early recovery can be related to a direct exchange between Pi and Pme both during exercise and recovery rather than to a temporary loss of the NMR visible pool of inorganic phosphate.

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