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Heterogeneity of metabolic response to muscular exercise in humans

New criteria of invariance defined by in vivo phosphorus-31 NMR spectroscopy

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^{31}P NMR spectroscopy at 4.7 T has been used in vivo to follow metabolic changes associated with exercise and subsequent recovery in the forearm flexor digitorum superficialis muscle of 14 healthy volunteers. The muscle content in phosphomonoesters at rest provides an index of glycogenolytic activity. Quantitative linear correlations have been shown to link end-of-exercise acidosis to recovery kinetics of phosphocreatine and phosphocreatine/organic phosphate ratio. These linear relationships constitute new metabolic invariants to be used in the study of myopathies and muscle adaptation to exercise.

NMR spectroscopy, in vivo; Phosphorylated metabolite; Intracellular acidosis; Human muscle

1. INTRODUCTION

Over the past decade ^{31}P NMR spectroscopy has been increasingly used to characterize the normal and pathological metabolic response accompanying muscle exercise. A number of studies has been devoted to the determination of features of muscle metabolism which remain invariant in healthy subjects [1-6], in order to provide baseline information for NMR studies of muscle diseases or muscle adaptation to exercise [7-18]. A consensual observation pertains to the qualitative link between the extent of intracellular acidosis at the end of exercise and the kinetics of recovery of the phosphorylated metabolites (such as ATP, P_i and phosphocreatine) consumed or generated during exercise. The purpose of this study is (**1**) to investigate the biochemical heterogeneity of muscular metabolic response to exercise on the basis of parameters recorded in vivo by ^{31}P NMR spectroscopy and (**2**) to quantitate the relationship between end-of-exercise acidosis and recovery kinetics of selected metabolic parameters (concentrations and ratios of concentrations).

2. MATERIALS AND METHODS

2.1. *Subjects*

The study was conducted on the dominant forearm of 14 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.

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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-turned 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.

2.4. *Data analysis*

Relative concentrations of metabolites were determined by integration of the respective resonances. They were expressed as percent of the PCr content at rest. 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), P_i (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 P_i, 1.27 for ATP and 1.63 for phosphomonoesters (PME). Intracellular pH was calculated from the chemical shift of P_i relative to PCr at −2.45 ppm with respect to 85% H_3PO_4 [19]. Values of PCr/P_i, PCr/ATP and PME at rest were averaged over the 3 spectra recorded during the first

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Table I

Metabolic parameters measured by ^{31}P NMR spectroscopy on human forearm muscle subjected to a rest-exercise-recovery protocol.

	Group I	Group II	Group III	Mean value
Rest				
pHr	6.99 ± 0.02	7.01 ± 0.01	7.00 ± 0.02	7.00 ± 0.02
PCr/P_i	9.24 ± 0.75	9.57 ± 0.37	8.09 ± 1.27	9.07 ± 0.94
PCr/ATP	4.09 ± 0.57	4.00 ± 0.67	3.83 ± 0.20	4.02 ± 0.60
PMEr	2.88 ± 0.23*	3.7 ± 0.34*	5.00 ± 0.1*	
End-of-exercise				
pH_e	6.78 ± 0.05*	6.62 ± 0.04*	6.33 ± 0.07*	
PCr/P_i	1.74 ± 0.39	1.64 ± 0.06	1.11 ± 0.10	
PCr	50.43 ± 9.46	61.96 ± 6.03	41.4 ± 13.4	
Recovery				
d(PCr)dt	12.15 ± 0.51*	10.6 ± 0.58*	8.66 ± 1.1*	
$t_{1/2}$ PCr	1.46 ± 0.14*	1.93 ± 0.11*	2.8 ± 0.20*	
$d(PCr/P_i)/dt$	3.82 ± 0.62*	3.25 ± 0.06*	1.52 ± 0.60*	

Results are presented as mean ± SD. *indicates significant unpaired t-test ($P<0.01$). PCr values are expressed as percent of the resting content. $d(PCr/P_i)/dt$ and $d(PCr)/dt$ are respectively expressed as PCr/P_i units per min and percent of PCr per min. $t_{1/2}$ PCr (half-time of PCr recovery) is in min

3 min of the protocol. Time-dependent recovery profiles of PCr, P_i and PCr/P_i are assumed to be described by straight lines during the 4 first min following the end of exercise. Results are presented as mean ± SD. Unpaired t-tests were computed for all parameters in Table I with $P<0.01$ considered to be significant.

3. RESULTS

The 14 healthy subjects have been separated into 3 experimental groups according to the values of intracellular pH which they reached at the end of exercise (pH_e). pH_e values ranged from 6.78 ± 0.05 (group I, 8 subjects) to 6.33 ± 0.07 (group III, 3 subjects) with an intermediate value at 6.62 ± 0.04 (group II, 3 subjets).

At rest due to the increased spectral resolution afforded at 4.7 T, we were able to accurately measure the P_i and PME levels. As indicated in Table I, no significant differences were observed among the 3 groups for Pcr/P_i, PCr/ATP and intracellular pH. Conversely, the resting level of PME differed in a ratio of 1:2 between groups I and III. As shown in Fig. 1, a linear correlation between end-of-exercise acidosis (pH_e) and PME level at rest can be calculated.

During exercise, the expected decrease in PCr level with the symmetrical increase in P_i were observed. Concurrently the upfield shift of the P_i signal reflected intracellular acidosis. Some changes in the width and shape of the P_i signal were occasionally apparent. In a few cases, a splitting into 2 resolved P_i signals was observed. The PME resonance increased, appearing as a broad signal never exceeding the P_i resonance. This increase brought the PME level to about 4 times the resting value with no relationship to the extent of intracellular acidosis. Homeostasis of ATP was maintained throughout the exercise protocol. The extent of PCr breakdown, intracellular acidosis as well as values of PCr/P_i varied among the subjects (Table I). No significant relationship between PCr breakdown or PCr/P_i and pH_e was found.

During recovery following 3 min of exercise, the kinetic profile of the rapid build-up of PCr was well described by a single exponential for all subjects. The return of P_i to pre-exercise level was always faster than that of PCr. After 3 min of recovery, PCr was back to about 80% of its resting concentration while P_i was already reduced below its resting level [20]. At the same time, PME were still high and remained so during the first 6 min of recovery. It is noteworthy that P_i reappearance was concurrent to the recovery of PME to its resting value [20]. Other recovery parameters are reported in Table I.

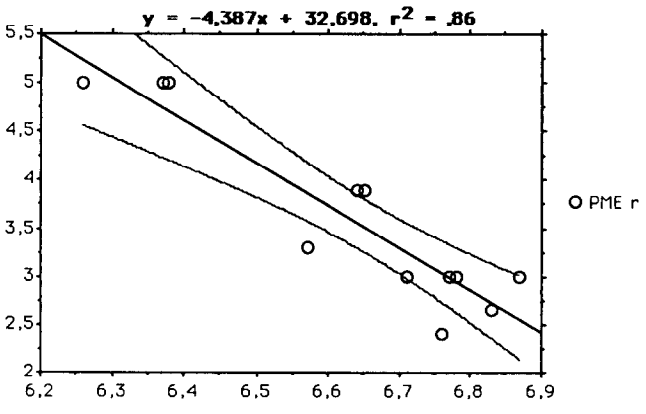

Fig. 1. Linear correlation between the PME resting content (PMEr) and the intracellular acidosis calculated at the end of exercise (pH_e). Dotted lines represent 99% confidence bands for the true mean of Y.

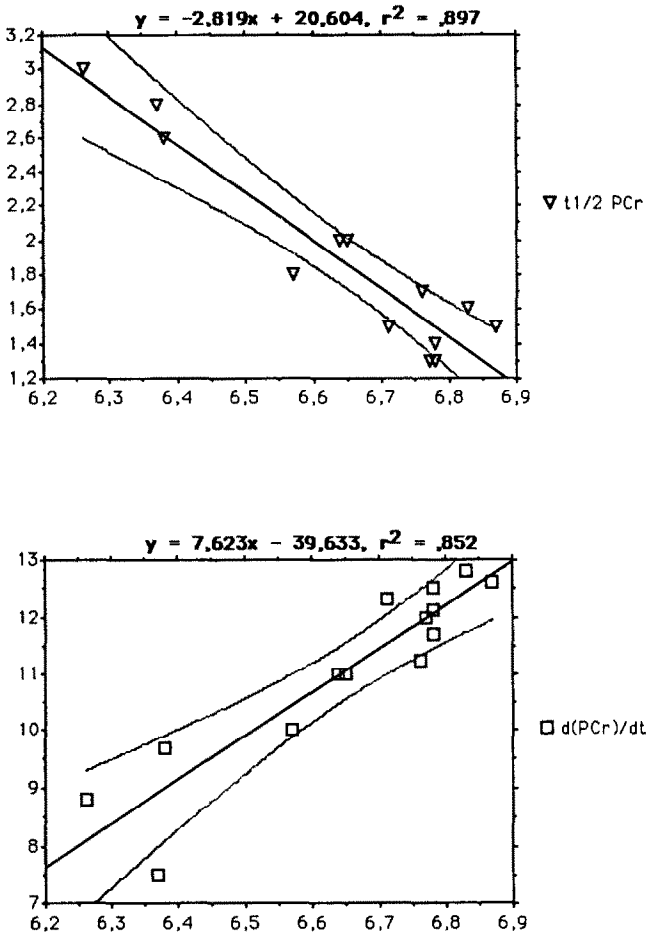

Fig. 2. Linear correlation between recovery kinetics of PCr (half-time recovery: $t_{1/2}$ PCr or d(PCr/dt)) and intracellular pH value at the end of exercise (pH_e). Dotted lintes represent 99% confidence bands for the true mean of Y.

The mean half-time ($t_{1/2}$ PCr) and the mean average rate (d(PCr)/dt) of PCr resynthesis, calculated during the initial 4 min of recovery can be correlated to end-of-exercise pH values (Fig. 2). Similarly, the mean average rate of Pcr/P_i ratio recovery (d(PCr/P_i/dt) linearly correlated with acidosis (Fig. 3). No correlation was found between the half-time or mean average rate (d(P_i/dt) of P_i recovery and pH_e. As expected [6], pH displayed a transient further decrease during the first min of recovery. Resting value was reached in 5 min.

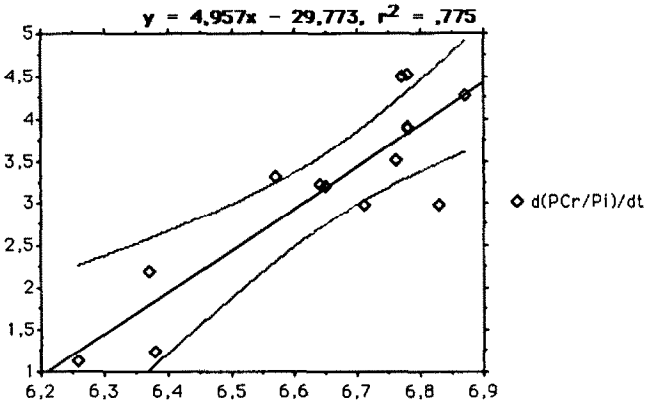

Fig. 3. Linear correlation between recovery kinetics of PCr/P_i ratio (d(PCr/P_i/dt)) and end-of-exercise acidosis (pH_e). Dotted lines represent 99% confidence bands for the true mean of Y.

Over the whole experimental course, the sum of all phosphorylated signals measured by NMR spectroscopy remained constant as did ATP. The stability of the signal-to-noise ratio during the 3 periods of the protocol is evidenced by the constancy of the intensity of the reference MDP signal. All parameters got back to their resting values in ca 10 min.

4. DISCUSSION

Large intersubject uncorrelated variations have been observed during exercise for metabolic parameters such as PCr breakdown, PCr/P_i and intracellular pH. These differences reflect different fiber types in the muscle volume 'seen' by the surface coil as well as variations in the distribution of these fibers due to intense training, daily low level physical activity or lack of any physical exercise [15–18]. In agreement with Arnold et al. [5], we have found that the extent of intracellular acidosis reached at the end of exercise determined the rate of PCr resynthesis. Further, we have established a direct linear relationship between pH_e and the initial kinetic parameters describing the recovery of PCr level and PCr/P_i ratio. These relationships characterized by high correlation coefficient (from 0.78 to 0.9) are independent from either the work output or the differential recruitment of glycolytic and oxidative fibers during exercise. Low intracellular pH values are known to induce a direct inhibition of ATP resynthesis by creatine kinase. Also, the rate-limiting step of glycolysis under the control of phosphofructokinase is inhibited at low pH. The effect of acidosis could then be in fine to reduce ATP available for PCr resynthesis [6–8].

Our finding that PME level at rest is significantly correlated with end-of-exercise acidosis supports that PME level at rest could be an index of the glycogenolytic capacity of the forearm flexor digitorum superficialis muscle. Again, the variations in the PME level that we have noted among the 3 groups of subjects may just reflect different distribution of fibers and training. Differences in metabolite contents at rest among fibers are well known [16]. Also, the forearm flexor muscles of elite runners contain more PCr, P_i and ATP than normal subjects [17]. We propose that, as in the case of PCr which is found at higher intracellular level and is more rapidly utilised in fast-twitch fibers, the PME content at rest constitutes a reliable proxy of the glycogenolytic activity of the muscle.

The 4 linear relationships reported in this paper for healthy subjects provide a new basis and new invariant parameters for the quantitative in vivo study by ^{31}P

NMR spectroscopy of muscle diseases as well as muscle adaptation to exercise. These relationships have been already tested and used successfully in our laboratory in the study of over 200 cases of human metabolic myopathies.

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