Intersubject differences in the effect of acidosis on phosphocreatine recovery kinetics after exercise

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Introduction

Traditionally, oxidative capacity of human skeletal muscle has been determined by *in vitro* analysis of maximal activity of marker oxidative enzymes in muscle biopsies or *in vivo* measurement of maximal whole body oxygen consumption. ^{31}P magnetic resonance spectroscopy (MRS) offers a non-invasive alternative to measure oxidative capacity. During recovery from exercise, phosphocreatine (PCr) is resynthesized purely as a consequence of oxidative ATP synthesis and therefore analysis of PCr recovery provides information about mitochondrial function. Several studies have shown that cytosolic pH has a strong influence on the kinetics of PCr recovery [1,2]. It has been suggested that PCr recovery time constants normalized for pH are a more accurate measure of oxidative capacity. However, a general correction for pH can only be made if there are no intersubject differences in the pH dependence of PCr recovery kinetics. We investigated the effect of acidosis on PCr recovery on a subject-by-subject basis.

Materials & Methods

Five subjects participated in the study (3 male, 2 female, age: 26 ± 6 years). ^{31}P MRS was performed by using a 1.5-Tesla whole-body magnet (Gyroscan S15/ACS, Philips Medical Systems, Best, the Netherlands) and a 6-cm diameter surface coil placed over the *M. vastus lateralis*. Spectra were acquired using a repetition time of 3 s and 2 scans per spectrum (6 s time resolution) during a rest-exercise-recovery protocol. All the subjects performed a single-leg extension exercise. One contraction was performed every 1.5 s. The workload was set at 7.5 W for the first min and then increased by 5 W each min. To achieve different levels of metabolic activation, and hence different degrees of cytosolic acidification, subjects performed exercises of different duration. Each subject performed 10-13 different protocols during 4-9 different sessions in a randomised order, with at least 15 min rest between different protocols within one session.

PCr, inorganic phosphate (P_i) and ATP signals were fitted in the time domain by using a non-linear least squares algorithm (AMARES) in the jMRUI software package. Absolute concentrations of the phosphorylated metabolites were calculated after correction for partial saturation and assuming that [ATP] is 8.2 mM at rest. Intracellular pH was calculated from the chemical shift difference between the P_i and PCr resonances. Free cytosolic [ADP] was calculated from pH and [PCr] using a creatine kinase (CK) equilibrium constant of 1.66×10^9 M^{-1} and assuming that 15% of the total creatine is unphosphorylated at rest. Recoveries of PCr and ADP were fitted to mono-exponential functions. Results are expressed as the metabolite's time constant of recovery, i.e. τ_{PCr} and τ_{ADP}. The initial PCr recovery rate (V_{PCr}) and maximum aerobic capacity (Q_{max}) were calculated as described by Kemp *et al.* [3].

Results

Figure 1 illustrates both the raw data and mono-exponential fits of the PCr and ADP recoveries from one measurement. For each subject, there is a negative linear relationship between τ_{PCr} and the end-exercise pH (Table 1; average R = -0.93 ± 0.06). Figure 2 shows the results for three of the subjects. Around pH 7 the τ_{PCr} is very similar for all subjects, but the pH dependence of τ_{PCr} differs, with some subjects showing a stronger pH dependence than others (Table 1 and Figure 2). The post-exercise ADP recovery is faster than the PCr recovery. On average, τ_{ADP} does not depend on the end-exercise pH (R = 0.54 ± 0.30) and for some subjects τ_{ADP} is even positively correlated with the end-exercise pH (e.g. subject 1, Figure 2). V_{PCr} and Q_{max} are also independent of the end-exercise pH (R = 0.24 ± 0.40 and R = 0.55 ± 0.31, respectively).

Discussion

Does the slower PCr recovery in the presence of intracellular acidosis reflect a decreased mitochondrial respiration at low pH, or is the PCr recovery slowed down due to factors downstream of oxidative phosphorylation? Because τ_{ADP}, V_{PCr} and Q_{max} are independent of the end-exercise pH, it is tempting to speculate that mitochondrial respiration is not decreased by intracellular acidosis and that the pH dependence of τ_{PCr} is caused by a decreased shuttling of ATP through the CK reaction and/or a pH-dependent shift in the CK equilibrium. The observed intersubject differences in the pH dependence of τ_{PCr} are likely to reflect differences in the rate of pH recovery, e.g. due to a more or less efficient proton efflux.

Conclusion

The pH dependence of τ_{PCr} differs among subjects and therefore no general formula can be applied to correct the τ_{PCr} for differences in end-exercise pH.

Table 1 Linear regression of τ_{PCr} and the end-exercise pH for the five subjects: correlation coefficient (R), slope and τ_{PCr} at pH 7 calculated from the linear relation.

	subject				
	1 (n=10)	**2** (n=12)	**3** (n=13)	**4** (n=12)	**5** (n=13)
R	-0.99	-0.98	-0.94	-0.87	-0.85
slope (s/pH unit)	-42.0	-58.3	-74.5	-56.9	-33.2
τ_{PCr} at pH 7 (s)	25.5	22.6	26.6	26.6	28.8

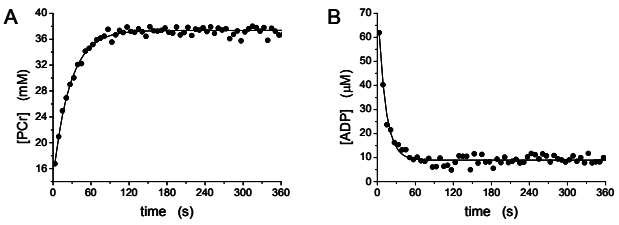

Figure 1 PCr (A) and ADP (B) recovery curves for an individual subject. Mono-exponential functions (dark lines) were fit to the actual data (filled circles).

References

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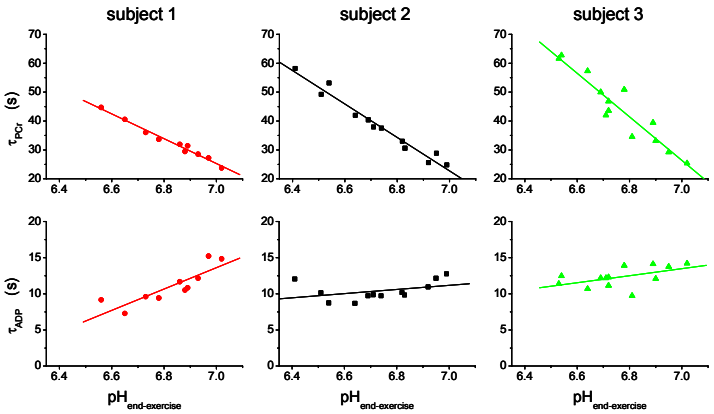

Figure 2 Correlation of τ_{PCr} (upper panels) and τ_{ADP} (lower panels) with the end-exercise pH for three different subjects. Linear functions (lines) were fit to the actual data (symbols).