Intersubject differences in the effect of acidosis on phosphocreatine recovery kinetics after exercise are due to differences in proton efflux rates

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Introduction

^{31}P MRS provides the possibility to obtain bio-energetic data during skeletal muscle exercise and recovery in a non-invasive manner and with a time resolution of a few seconds. During recovery from exercise, phosphocreatine (PCr) is resynthesized purely as a consequence of oxidative ATP synthesis and therefore the time constant of PCr recovery (τ_{PCr}) provides information about mitochondrial function. Several studies have shown that cytosolic pH has a strong influence on the kinetics of PCr recovery [1,2] and it has been suggested that τ_{PCr} normalized for pH is a more accurate measure of mitochondrial function. 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 pH dependence of PCr recovery on a subject-by-subject basis. Furthermore, we determined the kinetics of proton efflux at the start of recovery to obtain a measure of the rate of pH recovery.

Materials & Methods

Six healthy subjects participated in the study (4 male, 2 female, age: 30.8 ± 12.1 years). ^{31}P MRS was performed by using a 1.5-Tesla whole-body scanner (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 7.5-12.5 W for the first min and was 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 randomized 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. The recovery of PCr was fitted to a mono-exponential function yielding the time constant of PCr recovery: τ_{PCr}. The proton efflux rate E and the apparent efflux rate constant λ at the start of recovery were calculated from the changes in pH and PCr concentration during the first 12 s of recovery [3].

Results

Figure 1 illustrates both the raw data and mono-exponential fit of the PCr recovery from one measurement. For each subject, there was a strong negative linear relationship between τ_{PCr} and the end-exercise pH (pH_{end}). Figure 2 shows the correlation between τ_{PCr} and pH_{end} for three of the subjects. Around pH_{end} 7 τ_{PCr} was very similar for these three subjects, but at lower pH_{end} values τ_{PCr} differed. Therefore, the pH dependence of τ_{PCr} differed, with subject 1 showing the weakest pH dependence and subject 3 showing the strongest pH dependence. The results of the linear regression analyses for all subjects are shown in Table 1. The slope of the relation between τ_{PCr} and pH_{end} ranged from -33.0 to -75.3 s per pH unit. For subject 5, the proton efflux rate and the apparent efflux rate constant could not be determined. For the other five subjects, the mean proton efflux rate E was 16 ± 3 mM/min and the mean apparent efflux rate constant λ was 38 ± 6 mM/(min · pH unit). In Figures 3a and 3b, the slope of the relation between τ_{PCr} and pH_{end} is plotted against E and λ, respectively. The slope of the relation between τ_{PCr} and pH_{end} was positively correlated with both E ($R = 0.91$, $p = 0.03$) and λ ($R = 0.96$, $p = 0.01$).

Discussion

Intracellular acidosis slowed PCr recovery and the pH dependence of τ_{PCr} differed among subjects, ranging from -33.0 to -75.3 s per pH unit. The observed intersubject differences in the pH dependence of τ_{PCr} are likely to reflect differences in the rate of pH recovery. Unfortunately, the recovery of pH could not be investigated, because the P_i peak consistently disappeared within the noise after about 1 min of recovery and for the exercises at higher intensities was not fully recovered by the end of the time series. The recovery of cytosolic pH to the resting value is a function of net proton efflux. We calculated proton efflux rates E and apparent proton efflux rate constants λ at the start of recovery. The slope of the relation between τ_{PCr} and pH_{end} was positively correlated with both E and λ, indicating that subjects with a smaller pH dependence of τ_{PCr} have a higher proton efflux rate. Higher proton efflux rates will lead to faster pH recovery and therefore the observed correlations support our hypothesis that the intersubject differences in the pH dependence of τ_{PCr} are caused by differences in the rate of pH recovery. Our study implies that simply correcting τ_{PCr} for end-exercise pH is not adequate, in particular when comparing patient and controls, as certain disorders are characterized by altered proton efflux from muscle fibers. Also, matching for end-exercise pH is not sufficient when subject groups systematically differ in proton efflux kinetics.

Conclusion

Intersubject differences in the effect of acidosis on PCr recovery kinetics after exercise are due to differences in proton efflux rates. Simply correcting τ_{PCr} for end-exercise pH is not adequate and τ_{PCr} can only be used as a measure of mitochondrial function when end-exercise pH is close to resting values.

References

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Table 1 Linear regression of τ_{PCr} and pH_{end} for the six subjects.

subject	R	slope (s/U)
1 (n=10)	-0.99	-42.9 ± 2.1
2 (n=12)	-0.98	-57.9 ± 3.5
3 (n=13)	-0.94	-75.3 ± 8.1
4 (n=12)	-0.87	-56.2 ± 9.9
5 (n=13)	-0.85	-33.0 ± 6.3
6 (n=11)	-0.94	-62.9 ± 7.7

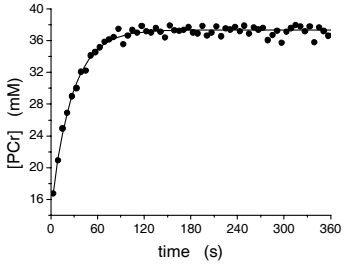

Figure 1 PCr recovery curve for one subject. A mono-exponential function (dark lines) was fit to the actual data (filled circles) obtained every 6 s. The time constant for PCr recovery was 26.9 s.

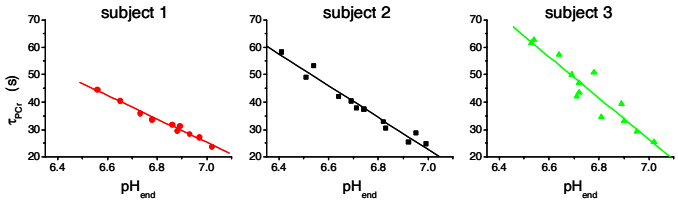

Figure 2 Correlation of τ_{PCr} with pH_{end} for three different subjects. Linear functions (lines) were fit to the actual data (symbols).

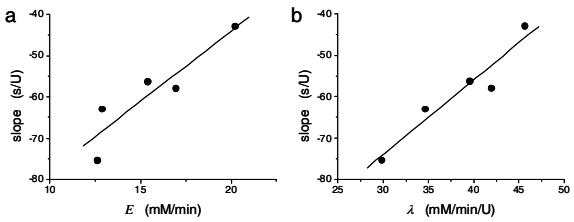

Figure 3 Correlations between the slope of the relation between τ_{PCr} and pH_{end} and (panel a) the proton efflux rate E ($R = 0.91$, $p = 0.03$) and (panel b) the apparent efflux rate constant λ ($R = 0.96$, $p = 0.01$) for five of the six subjects.