Exercise protocol and muscular fiber type composition dependent phosphocreatine recovery in health and disease

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Introduction

^{31}P-MRS provides means to obtain bioenergetic data from skeletal muscle during exercise and recovery. The time constant (TC) of phosphocreatine (PCr) recovery is used as a measure of mitochondrial function. Muscle fibers are either slow twitch (ST) oxidative or fast twitch (FT) glycolytic fibers. While ST fibers are generally recruited at lower force levels than FT fibers, at high contraction-velocities both types of fibers are recruited already at low force levels [1,2]. This differential fiber type recruitment legitimates the distinction between exercise protocols of both low and increasing intensity at low contraction frequency (LE), and exercise protocols of high intensity and/or high contraction frequency (HE). Utilizing LE protocols, patients with mitochondrial encephalomyopathies (ME) and patients with migraine with aura (MA) have been demonstrated to differ from healthy volunteers (HV) with respect to TC values normalized for minimum pH reached during recovery (pH_{min}) [3,4].

In this work, we show that the dependence of fiber type recruitment on contraction speed and exercise intensity may produce different pH_{min} and TC values contingent on the employed exercise protocol in cooperation with the individual fiber type composition of the muscle under investigation. Thus, it might be hypothesized that differences between HV, MA and ME patients in muscle fiber composition and recruitment as well as in proton efflux rate, which influences the pH_{min} and is known to be higher in ME patients, might mask the mitochondrial impairment in the patient groups when using a HE protocol instead of a LE protocol to investigate muscle energy metabolism with in vivo ^{31}P-MRS. Therefore it can be also assumed that training, which changes muscle fiber type composition [2], might have an influence on TC and pH_{min} values. In order to support the above hypotheses a high contraction-velocity submaximal exercise protocol that fully activates all muscle fibers investigated was applied to HV, ME patients and MA patients.

Materials and Methods

Controls and patients: 21 HV (12 females (f), 9 males (m); age: 18-64 years), 5 MA (4 f, 1 m; age: 18-37 years) and 6 ME (3 f, 3 m; age: 28-58 years) were included into the analysis. All examinations were carried out according to the standards of the Declaration of Helsinki and were approved by the ethics committee Zürich.

Exercise protocol: In-magnet exercise was performed by pressing a foot-pedal. Resistance of the pedal was given by a blood pressure cuff. The subject being examined pressed the pedal at a frequency of 80/min, triggered by a metronome. Each plantar flexion was executed as rapidly as possible.

^{31}P-MRS: Spectra were acquired on a 1.5 T Philips Intera human MR system with a 10 cm diameter single-tuned circular transmit/receive surface coil positioned on the right calf muscle using a pulse-and-acquire technique. Excitation was carried out by a B1 insensitive rotation (BIR-4) pulse set to a flip angle of 40 degrees, using a receiver bandwidth of 1500 Hz. A time series of 100 free induction decays (FIDs) with 512 samples per FID was recorded, using the following 10 min paradigm: 2 min (20 spectra) resting phase, 2 min (20 spectra) exercise phase, 6 min (60 spectra) recovery phase. Each time step consisted of 4 averages with a repetition time of 1.5 s each. The shim volume was chosen to fit the anatomy of the subject and was roughly 80ml.

Data analysis: Using jMRUI v2.2 zero-order phase correction was applied manually, a 9.8 Hz apodization filter and direct current (DC) correction were executed on the time series. Quantification was done with jMRUI's AMARES algorithm. A best fit to the experimental points was calculated using Solver in Excel 2002. Statistical analysis included checking the data for normal distribution, quantifying the differences between the groups with the effect size index δ and testing for significance with ANOVA using SPSS 17.0. Linear regression analysis (SPSS 17.0) was used to model the relationship between the TC value and pH_{min}.

Results

TC values extracted from the monoexponential fits plotted against pH_{min} are reported for all subjects in Figure 1a. All data, including patient data, lie within the 95% individual prediction interval of the regression line (95-PI) (correlation coefficient r=−0.56) linking the TC values of PCr recovery to the pH_{min} values of the HV. Taking into account the non-monoexponential recovery of PCr at pH <6.50 [5] and additionally excluding ten HV data sets – for reasons of age older than 60 years since oxidative capacity has been revealed to decrease with age by 50% in elderly (65-80 years) healthy subjects [6], not reaching near steady state level of PCr at the end of exercise and endurance training – resulted in a clearly improved correlation coefficient r=−0.77 for the control group. Two ME and one MA data point come to lie outside of the 95-PI (Figure 1b).

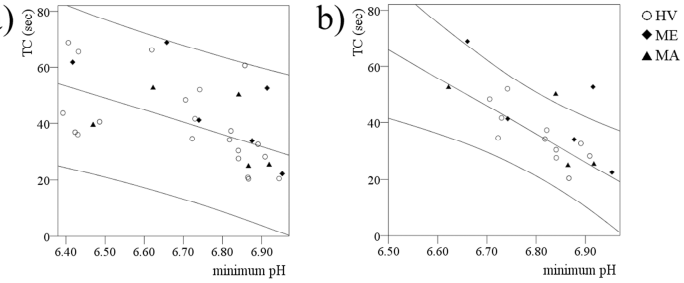

Figure 1: Phosphocreatine (PCr) recovery time plotted against minimum pH (pH_{min}) *without exclusions* in a) compared to *with exclusions* in b); equation for regression line and r value;

a) 21 HV, 6 ME and 5 MA; y=−43·x+329, r=−0.56; all recorded data lie within the 95% individual prediction interval of HV;

b) pH_{min} >6.50: after exclusion of 10 HV: 11 HV, 5 ME, 4 MA; y=−99·x+710, r=−0.77; 2 ME and 1 MA data point lie outside of the 95% individual prediction interval of HV;

The PCr recovery time is represented by the time constant (TC) of the monoexponential function best fitting the experimental points and is plotted as a function of pH_{min} which refers to the lowest value of cytosolic pH reached during the first minute of recovery after exercise. The central straight line in each plot illustrates the linear regression linking the TC values to the pH_{min} values of the HV and the two outer lines delimit the corresponding 95% individual prediction interval.

Discussion

It seems likely that the unambiguous differences between groups of healthy controls and ME patients observed in previous studies employing LE protocols on gastrocnemius muscle result from activation of higher fractions of FT fibers in muscles of ME patients in comparison to HV, rather than from truly comparing whole muscle oxidative capacities. Thus, when employing HE protocols, the activation pattern of muscle fibers in HV and ME patients and the interindividual variations in muscle fiber type composition in HV mask the differences between controls and ME patients detected earlier with LE protocols. Our results also point to the importance of careful matching of the groups regarding age and activity level when looking for differences between controls and ME and even MA.

In conclusion, our observations challenge the notion of absolute workload independence of PCr recovery rates [7,8] and suggest a dependence on exercise protocols and individual muscle fiber type composition due to different PCr recovery characteristics of FT and ST muscle fibers.

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