An In-Vivo Examination of Skeletal Muscle Water Energetics Post Exercise Using NMR Relaxomety, Multi-Frequency Bio-impedance Analysis and 31P Spectroscopy

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Synopsis: Using NMR relaxometry we previously have shown that five T_2 components could be resolved, *in vivo*, for muscle water. The three components with $T_2 <$ 100 ms were tentatively assigned to intracellular water (ICW_{NMR}). In this study, we have utilized multi-frequency bio-impedance (MFBIA) as an independent measure of ICW and compared this with ICW_{NMR} at the end of exhaustive exercise and during recovery. No significant differences were found between short-term recovery of ICW_{NMR} and ICW_{MFBIA}. Furthermore using ^{31}P spectroscopy, significant correlations were found between increases in ICW and pH at the end of exercise.

Introduction:

Changes in transverse relaxation (T_2) of skeletal muscle have been studied extensively using imaging techniques, typically yielding a single T_2 value that increases with intense exercise (3). Using an NMR relaxometry technique, multi-exponential analysis of skeletal muscle T_2 revealed as many as five components (designated A, B, C, D and E in order of increasing T_2) (7). Furthermore, following exhaustive exercise, the magnitude of A+B+C increased significantly (8). The authors theorized that changes in signal from these components ($T_2 <$ 100 msec; comprising of ~85% of the total signal) likely reflected changes in intracellular water.

The purpose of this study was twofold. First, relaxometry-measured changes in multi-component T_2 were compared to changes in ICW_{MFBIA} during short-term recovery from exhaustive exercise in order to investigate if our assignment of the components with $T_2 <$ 100 ms to ICW was consistent with the independently measured ICW_{MFBIA}. Secondly, using ^{31}P spectroscopy, we attempted to elucidate whether any significant relationships exist between changes in pH and Pi/PCr and changes in ICW concentration following exercise.

Methods:

Methods were approved by the University of Western Ontario Ethics Review Board. Seven healthy men (age 26 ± 2 years old) performed an exhaustive wrist flexion exercise (0.5 Hz contractions) for all three experimental conditions (NMR, MFBIA, ^{31}P). Measurements were taken at rest, end exercise and during 15 minutes of recovery. ^{1}H NMR data were acquired using a 21-cm bore, 1.89 T magnet interfaced to a SMIS/IMRIS console. All data was collected from the flexor digitorium profundus (FDP) muscle using an *in-vivo* relaxometry technique (projection presaturation-CPMG) described in detail elsewhere (7). The relaxometry data were analyzed with the non-negative least squares algorithm (9).

MFBIA measurements of the forearm were performed using a SEAC model SFB3 (Uniquest, St. Lucia Queensland). Electrodes were placed in a tetrapolar arrangement with the drive electrodes (3M Red Dot Ag/Au) placed one on the dominant hand and one near the elbow in the forearm. Sense electrodes were placed between these on the forearm, approximately 4 cm away from the drive electrodes to help eliminate surface conduction effects. Measurements were taken at multiple frequencies logarithmically spaced between 4 and 1024kHz, and the impedance measured was fit to a Cole-Cole plot (2). The values for the resistance at zero and infinite frequencies were calculated from the fit and used to estimate the intra, extra, and total water (10).

^{31}P spectroscopy data were acquired on the same system as the ^{1}H using a 4 cm diameter surface coil situated under the belly of the forearm. A 3 ms adiabatic pulse was used in acquiring free induction decay (FID) data, with a 12 µs delay time, spectral width of 4000 Hz and 2048 complex data points. The FID consisted of 6 averages over 36 s (TR = 6 seconds). Each FID was fit to a sum of decaying exponentials using FITMAN software developed in our laboratory.

ICW data were normalized to resting values and analyzed as a function of time post exercise. An ANOVA was employed for statistical analysis of the two curves. Pi/PCr and pH were also examined as a function of time post exercise and the recovery modeled to a mono-exponential. End exercise values of ICW_{NMR}, ICW_{BIA} and pH were normalized by dividing by work output squared (4).

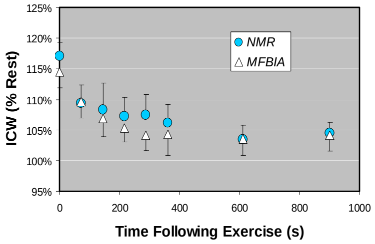

Fig. 1. Post-exercise recovery of ICW_{NMR} and ICW_{MFBIA}

Results:

During short-term recovery, no significant differences were found between ICW_{NMR} and ICW_{MFBIA} (fig. 1). Significant correlation was found for both normalized ICW_{NMR} and pH (r=0.88, p<0.05; fig. 2) and normalized ICW_{MFBIA} and pH (r=0.82, p<0.05) at the end of exercise.

Pi/PCr recovered quickly following exercise ($\tau_{Pi/PCr} <$ 100 s) while pH recovered slowly ($\tau_{pH} >$ 250 s). pH had not fully recovered to resting values by 900 s.

Discussion:

Lack of significant difference between ICW_{NMR} and ICW_{MFBIA} during recovery is consistent with the assignment of components A, B and C ($T_2 <$ 100 msec) to intracellular water. The observed increases in ICW (~15%) were larger than previously reported (~10%)(6). The exercise here was for a longer period of time and required recruitment of different forearm muscles. These factors may account for the higher increase in ICW. It is unlikely due to a systematic error as both ICW_{NMR} and ICW_{MFBIA} were increased.

The significant correlations between end exercise values of pH and ICW (as measured by both MFBIA and NMR) are in agreement with findings that suggest that intracellular acidification and other products of glycolysis may largely mediate increases in intracellular water observed in skeletal muscle during exercise (1).

ICW measured by both techniques appeared to recover bi-exponentially with a fast component (apparent $\tau <$ 120 s) and a slow component ($\tau >$ 300 s). At 900 s, ICW_{NMR} and ICW_{MFBIA} were still above baseline by 3-4%. This may suggest that the initial recovery of ICW following exercise is influenced by Pi/PCr and pH while the long-term recovery is influenced by pH.

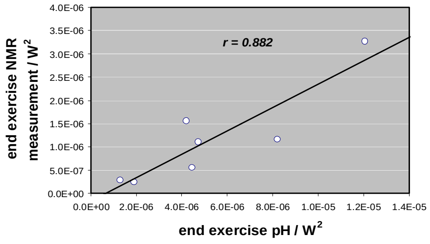

Fig. 2. Relationship between ICW_{NMR} and pH at end exercise (values normalized by dividing by square of work output)

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