In Vivo Bioenergetics of Rat Hindlimb Muscle After Moderate Spinal Cord Contusion


Introduction
Declines in skeletal muscle oxidative capacity following spinal cord injury (SCI) has the potential to decrease exercise capacity and negatively impact muscle fatigability (Bhambhani et al. 2000). Though altered oxidative capacity after SCI appears to be well documented, most investigators have utilized in vitro measurement techniques in their studies. $^{31}$P MRS offers a unique non-invasive alternative of measuring oxidative capacity of skeletal muscle and is especially suitable for longitudinal investigations. The purpose of this study was to determine the impact of spinal cord contusion on the oxidative capacity of rat hindlimb using $^{31}$P MRS.

Experiment Methods
Five young adult female rats (age 16 weeks) were moderately injured at the T8-T10 thoracic spinal cord. $^{31}$P magnetic resonance spectroscopic ($^{31}$P-MRS) measurements were performed at weekly intervals for assessments of oxidative capacity of the rat hindlimb muscle for three weeks. Spectra were acquired in a Bruker 11T/470 MHz spectrometer using a $^{31}$P (190.5 MHz) surface coil, placed over the belly of the gastrocnemius muscles. Standard $^1$H surface coil was placed underneath the hindlimb to perform shimming. The sciatic nerve was electrically stimulated by subcutaneous needle electrodes with a frequency of 1 Hz and a 1 ms duration. Spectra were acquired with a 50 µs square pulse, a TR of 2 s, sweep width of 10,000 Hz and 8,000 complex data points. Phosphorus spectra were collected in 30 s bins at rest (5 min), during stimulation (6 min), and recovery (20 min) (Fig 1).

Spectral analysis: The spectra were manually phased, and the areas of the γ-ATP, P$_i$, and PCr peaks were determined using area integration. The PCr area during recovery was fit to a single exponential curve, and the pseudo-first-order rate constant for PCr recovery ($k_{PCr}$) was determined (Meyer 1988).

Data analysis: Repeated measures ANOVA were used to compare $^{31}$P MRS measurements prior to and after SCI. Research hypotheses were tested at an alpha level of 0.05 and included post-hoc Bonferonni corrections.

Results and Discussion
Following SCI resting spectra demonstrated a significant increase in P$_i$/PCr ratio (~10%). As compared to control rats, spinal cord injured rats reached a similar PCr drop (~35% to 50%) following lesser durations of EMS (4 minutes as versus 6 minutes) demonstrating an increase in ATP demands in the injured group. Additionally, PCr recovery rates were significantly declined (p<0.008, 22%) after one week of SCI and regained within two weeks after the injury (Fig 2).

Conclusions
Our findings clearly reveal a reduction in the oxidative capacity of skeletal muscle as determined by PCr recovery rate in rat hindlimb after one week of spinal cord contusion. The aforementioned data indicate that muscle disuse accompanying SCI induces a decrease in the oxidative capacity of skeletal muscle which can be measured with $^{31}$P MRS.

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References