^{31}P Magnetic Resonance Spectroscopy of Mouse Skeletal Muscle Following Hind Limb Cast Immobilization

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INTRODUCTION

Apart from structural and morphological adaptations, disuse atrophy has shown to induce important metabolic adaptations in the skeletal muscle. A large number of *in vitro* studies have shown that there is a significant reduction in the maximal activity of essential oxidative enzymes following immobilization. ^{31}P MRS offers a unique non-invasive alternative to measure the *in vivo* oxidative capacity of skeletal muscle. The purpose of this study was to determine the impact of disuse atrophy on the *in vivo* oxidative capacity of mouse hindlimb muscles using ^{31}P MRS.

METHODS

Young adult mice (C57BL6 female, n = 8) aged 19-21 weeks were studied. The lower hindlimbs of all mice were immobilized for a 2-week period. ^{31}P MR data were acquired before and immediately following immobilization in a Bruker 11T spectrometer. Spectra were acquired using a 6-mm x 12-mm oblong ^{31}P (190.5 MHz) surface coil, placed over the belly of the gastrocnemius muscles. A 3-cm ^{1}H surface coil was placed underneath the hindlimb to adjust magnetic field homogeneity. An inflatable blood pressure cuff was positioned around the animal's thigh. Spectra were collected with a 50μs square pulse, a TR of 2s, sweep width of 10,000 Hz and 8,000 complex data points in 30s bins starting at rest (10 min), during ischemia (30 min), and throughout recovery (30 min). Resting spectra were manually phased, and the areas of the γ-ATP, P_i, and PCr peaks were determined using area integration and assuming a resting ATP concentration of 8.2 mmol/L following saturation correction. Dynamic changes in PCr levels were determined using complex principal component analysis (Elliott 1999). The pseudo-first-order rate constant for PCr recovery (k_{PCr}) was determined and used to calculate the *in vivo* oxidative capacity (Paganini 1997). Paired t tests were used for comparisons with an alpha level of 0.05.

RESULTS

Following immobilization resting ^{31}P MRS spectra were characterized by a significant increase in the basal P_i content (~70%; $p<0.001$) and the resting P_i/PCr ratio (~80%; $p< 0.001$). The resting PCr content and the intracellular pH values were not different (Table 1). During 30 minutes of ischemia, the PCr levels decreased by 45 to 55% while the ATP and pH values remained unchanged in both pre immobilized and immobilized muscles. No significant difference was noted in the rate of PCr depletion between pre immobilized versus immobilized muscles (0.45± 0.03 mM/min^{-1} vs. 0.52 ± 0.01 mM/min^{-1}; $p=0.78$). In contrast, immobilization induced a 30% decrease in the PCr recovery rate constant (0.45± 0.01 min^{-1} vs. 0.31± 0.01 min^{-1}; $p<0.001$; Fig. 2). This decrease in k_{PCr} reflects a decrease in the *in vivo* oxidative capacity of 5.4 mM ATP/min (~35%).

	PRE IM (rest)	POST IM (rest)	PRE IM (end ischemia)	POST IM (end ischemia)	
[P_i](mM)	2.64 ± 0.31	4.64 ± 0.41 *	7.32 ± 1.24	10.88 ± 1.42	*
[PCr] (mM)	34.68 ± 1.29	32.62 ± 1.05	18.67 ± 0.62	15.02 ± 0.79	*
P_i/PCr	0.08 ± 0.01	0.14 ± 0.01 *	0.39 ± 0.03	0.74 ± 0.10	*
pH	7.19 ± 0.02	7.17 ± 0.02	7.12 ± 0.02	7.05 ± 0.06	*

Table 1. Basal metabolite content and intracellular pH at rest and at the end of ischemia in C57 mice (n=8) prior to immobilization (PRE IM) and at 2 weeks of immobilization (POST IM). Values are mean ± S.E.M. * Statistically significant differences (p <0.05)

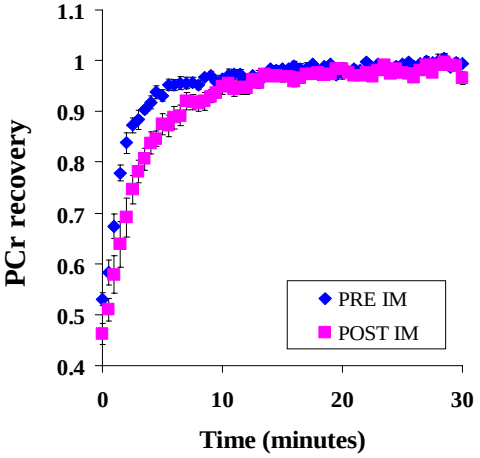

Fig. 2. PCr recovery kinetics in C57 mice (n=8) prior to immobilization (PRE IM) and following 2 weeks of immobilization (POST IM).

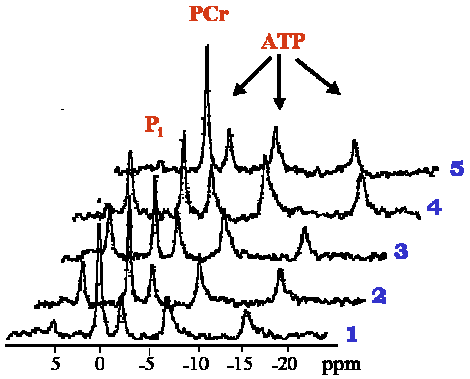

Fig. 1. ^{31}P spectra obtained from a pre immobilized C57 mouse at rest (1), after 10, 20, 30 min of ischemia (2-4) and after 10 min of recovery (5)

CONCLUSION

Cast immobilization induces significant metabolic adaptations in the lower hindlimb muscles. Based on the PCr recovery kinetics following ischemia we conclude that disuse atrophy induces a significant decrease in the *in vivo* oxidative capacity of skeletal muscle. Therefore therapeutic interventions following cast immobilization should include strategies to enhance mitochondrial function.

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