A quantitative study of bioenergetics in skeletal muscle lacking carbonic anhydrase III by 31P magnetic resonance spectroscopy

M. Liu[1], G. Walter[2], N. Pathare[1], U-J. Zimmerman[3], R. E. Forster[3], K. Vandenborne[1]

[1]Department of Physical Therapy, University of Florida, Gainesville, FL, United States, [2]Department of physiology and functional genomics, University of Florida, Gainesville, FL, United States, [3]Department of Physiology, University of Pennsylvania, Philadelphia, PA, United States

INTRODUCTION:

The purpose of this study was to provide *in vivo* experimental evidence for the biological importance of carbonic anhydrase (CA) III in energy metabolism of skeletal muscle. The CA gene family plays an important role in many physiological processes including renal acidification, respiration, and signal transduction. However, its role in skeletal muscle has not been well established. In order to assess quantitatively the importance of CA III in skeletal muscle, changes in phosphate metabolite concentrations and intracellular pH were measured in the hindlimb muscle of CA III knock out mice during and after electrical stimulation.

METHODS:

The MR measurements were performed inside an 11T/470 MHz spectrometer. Spectra were acquired using a 6-mm x 12-mm oblong phosphorus (190.5 MHz) surface coil, placed over the belly of the gastrocnemius muscles. A one turned standard ^{1}H coil was placed underneath the hindlimb to perform localized shimming. The lower hindlimb muscles were stimulated with a computer-activated Grass S-48 stimulator via bipolar electrodes placed adjacent to the sciatic nerve. All contractions were elicited using a series of intermittent stimulation trains at supramaximal voltages (~10 V), with a single train of 700ms duration at 100 Hz. Stimulus trains were given at a frequency of 1 Hz. Spectra were acquired with a 50 μs square pulse, a pulse repetition time of 2 sec, and data were averaged into 30 second bins. The sweep width was 10,000 Hz and 8,000 complex data points were used. Phosphorus spectra were obtained at rest (5 min), during electrical stimulation (2 min), and the following recovery (30 min). Intracellular pH was calculated based on the chemical shift of Pi. The Pi and PCr concentrations were determined using area integration. The pseudo-first-order rate constant for PCr recovery (k_{PCr}) was determined. At the termination of the NMR experiment, the gastrocnemius muscles were dissected and frozen in liquid nitrogen. The ATP concentration was determined using an ATP assay kit (Sigma).

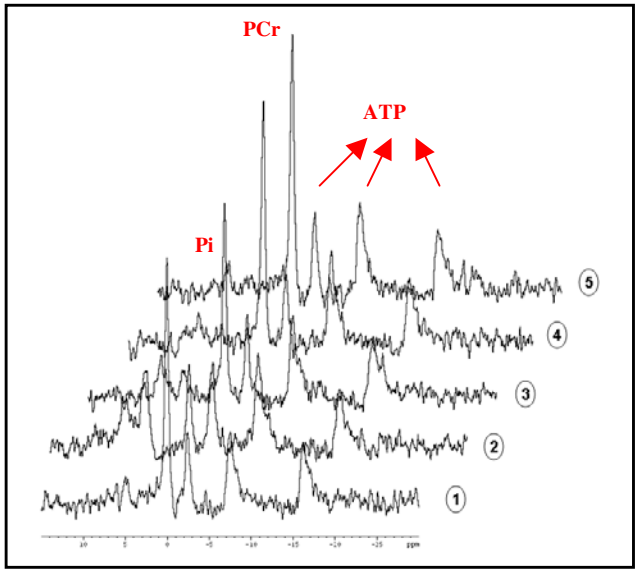

Figure 1: ^{31}P spectra obtained from a CA III knockout mouse at rest (1), after 2 min of electrical stimulation (2) and 30 min of recovery (3-5)

RESULTS:

^{31}P spectral analysis of skeletal muscle on CA III knock mutants (n=8) showed a PCr concentration of 31.55±0.94 mM and Pi concentration of 2.61±0.13 mM at rest. The resting intercellular pH was 7.19±0.02 (Table). During 2 minutes of ischemia, the PCr levels decreased by 85 to 90% while the pH dropped to 6.56±0.02. Following ischemia, the PCr concentration returned to baseline levels within 30 minutes. Pi levels recovered concomitantly. Compared to control mice (129/SvJ, n=8), no significant differences were observed in the resting phosphate concentration, PCr depletion and pH kinetics during electrical stimulation (figure 2). However, CA III knock out mice showed a slower rate of PCr recovery, with a k_{PCr} of 0.18±0.01 min^{-1} in CA III knock out mutants and a k_{PCr} of 0.30±0.04 min^{-1} in the wild type mice (figure 3).

CONCLUSION:

Our quantitative study of muscle bioenergetics in CA III knockout mice showed a slower rate of PCr recovery rate following high intensive electrical stimulation. These data suggested that CA III may play an important role in maintaining the bioenergetics' capacity of skeletal muscle following high intensive activation.

ACKNOWLEDGEMENTS:

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Table: Basal metabolite content and intercellular pH at rest

Variable	Group	
	CA III knockout	Control (129/sv)
n	16	16
Pi (mM)	2.61±0.12	2.66±0.16
PCr (mM)	31.55±0.94	30.74±1.42
Pi/PCr	0.08±0.01	0.09±0.1
pHi	7.19±0.02	7.20±0.02

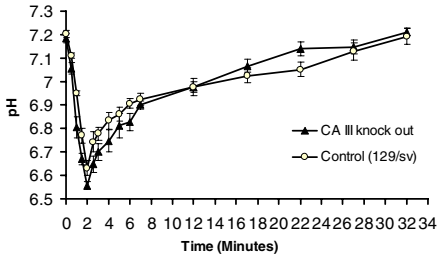

Figure 2: pH kinetics at rest (0 min), during electrical stimulation (0-2 min) and recovery from CA III knockout and wild type mice.

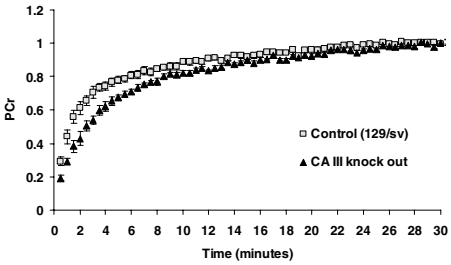

Figure 3: PCr kinetics during recovery in CA III knockout and wild type mice.