A Comparison of In Vivo and In Vitro Measurements of Skeletal Muscle Mitochondrial Capacity in Human Skeletal Muscle

I. R. Lanza[1], K. S. Nair[1], and J. D. Port[2]
[1]Endocrinology, Mayo Clinic, Rochester, Minnesota, United States, [2]Radiology, Mayo Clinic, Rochester, MN, United States

Introduction: Nearly 30 years ago, Britton Chance pioneered the application of phosphorous magnetic resonance spectroscopy (^{31}P-MRS) to monitor human skeletal muscle bioenergetics[1]. Skeletal muscle oxidative capacity can be determined from a kinetic analysis of the monoexponential rate of phosphocreatine (PCr) resynthesis following conditions that are known to deplete the intramyocellular PCr pool (e.g., electrical stimulation, voluntary muscle contraction, ischemia)[2]. Rapid technological advances have fueled a growing number of studies that have applied this methodology to assess mitochondrial capacity across a wide range of human studies, including children, elderly, athletes, and clinical populations characterized by mitochondrial impairments. This kinetic analysis of PCr recovery has been shown to be highly reproducible[3], but validity of this measurement is largely based on correlations with indirect markers of mitochondrial function such as maximal activities of key mitochondrial enzymes[4]. In the present study, we compared oxidative capacity determined by ^{31}P-MRS *in vivo* with 2 direct, independent measurements of mitochondrial oxidative capacity *in vitro* as a more robust assessment of the validity.

Methods: 11 healthy individuals (6 men, 5 women, age = 39±9 years) were recruited. Volunteers were given a standard weight-maintaining diet for 3 days prior to the study, provided by the metabolic kitchen at the Clinical Research Unit (CRU). Volunteers were admitted to the CRU for two consecutive days. *In vitro measurements:* On study day 1, following an overnight fast, a biopsy was performed on the vastus lateralis muscle and approximately 80mg was immediately used to isolate mitochondria by homogenization and differential centrifugation. Oxidative capacity of isolated mitochondria was determined by two methods. First, we measured the maximal rates of ATP synthesis using a bioluminescent method involving a luciferase light-emitting reaction using a luminometer (Turner Biosystems). Second, we measured the rates of oxygen consumption using a high-resolution polarographic oxygen electrode (Oxygraph 2K, Oroboros Instruments). The rates of ATP production and O_2 consumption were measured in the presence of glutamate and malate (substrates for respiratory chain complex I). All reactions were measured with 2.5mM ADP to stimulate maximal State 3 kinetics. *In vivo measurements:* On the second study day following an overnight fast, the contralateral leg was studied by ^{31}P-MRS to assess oxidative capacity in vivo. A ^{31}P surface coil (x cm diameter) was placed over the vastus lateralis muscle and the leg was positioned in the isocenter of a 3T GE Signa scanner. The leg was affixed to a custom-built device to measure isometric knee extension force in the magnet bore. Following manual shimming, baseline ^{31}P spectra were acquired using a nominal 90 degree hard pulse under partially saturated (2s TR) and fully relaxed (16s TR) conditions. Spectra were then continuously acquired during 72s of rest (6 dummy scans), 30 seconds of voluntary contraction of the knee extensor muscles, and 10 minutes of recovery. Subjects were instructed to forcefully contract their muscles while receiving visual feedback from a computer display. Millimolar concentrations of PCr, inorganic phosphate (Pi) and ATP were determined by peak integration and correction for partial saturation (Figure 1). Cellular pH was calculated from the chemical shift of Pi relative to PCr using a modified Henderson-Hasselbalch equation. A PCr recovery rate constant (K_{PCr}) was determined by a monoexponential fit, and oxidative capacity was calculated as K_{PCr}*[PCr]$_{rest}$.

Results: [PCr] decreased by approximately 20% on average during the 30s muscle contraction with a corresponding increase in [Pi] and slight alkalosis. Regression analyses revealed significant positive associations between V_{max} measured by ^{31}P-MRS and maximal respiration rates of isolated mitochondria (Figure 2). A weaker, but significant association was evident for V_{max} and maximal ATP synthesis rate with complex I substrates (Figure 3).

Conclusion: Skeletal muscle oxidative capacity measured by ^{31}P-MRS was well-correlated with two independent measurements of mitochondrial capacity performed using isolated mitochondria.

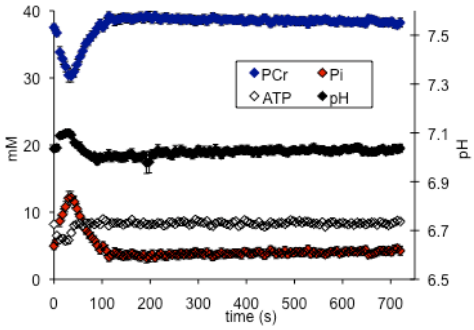

Figure 1: Mean [PCr], [Pi]. [ATP], and pH at rest, during 30 seconds of muscle contraction, and during 10 minutes of recovery

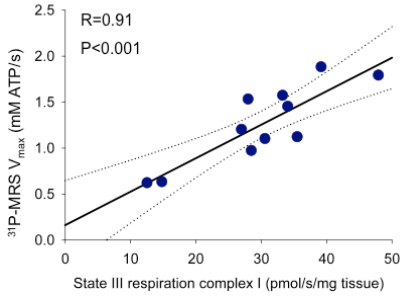

Figure 2: Oxidative capacity measure in vivo by ^{31}P-MRS was strongly correlated with maximal ADP-stimulated (state 3) respiration with glutamate + malate as substrates.

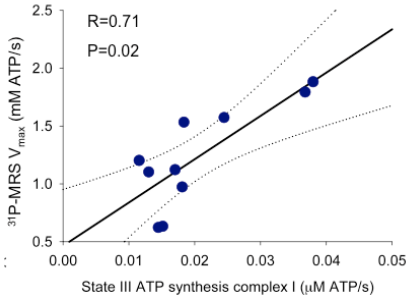

Figure 3: Oxidative capacity measure in vivo by ^{31}P-MRS was strongly correlated with maximal ATP synthesis rates with glutamate + malate as substrates.

References: 1. Chance B, PNAS, 77,1980. 2. Meyer RA, AJP, 254, 1988. 3. Layec G, Mag Res Med, 62, 2009 4. McCully K, JAP, 75, 1993