Resting and maximal oxidative ATP production are independent parameters of muscle mitochondrial function

D. E. Befroy[1], R. G. Larsen[2], M. A. Tevald[2], and J. A. Kent-Braun[2]
[1]Yale University, New Haven, CT, United States, [2]University of Massachusetts, Amherst, MA, United States

Introduction

A variety of MRS-based methods have been developed to estimate muscle mitochondrial function in vivo. Using these techniques, lower resting mitochondrial function and oxidative capacity have been observed in the muscle of elderly individuals, type 2 diabetics and their offspring. In contrast, several studies have observed no effects of aging or type-2 diabetes on mitochondrial capacity. The disparity in these findings is compounded by several factors: 1) each MRS method assesses a different characteristic of mitochondrial metabolism and it is unclear whether these are related or independent parameters of mitochondrial function, 2) muscle groups of different metabolic phenotype were investigated, and 3) group characteristics varied between studies. To address these issues, we have examined three ^{31}P-MRS techniques for estimating muscle mitochondrial metabolism in vivo in a single muscle compartment: resting muscle $P_i \rightarrow$ ATP flux, ischemic phosphocreatine (PCr) decline and post-contraction PCr recovery.

Methods

Eight healthy men, aged 21 to 34 years, were studied following a standard meal and 8 hour fast. All experiments were performed on a 4T Bruker Medspec system, using an elliptical 3 x 4cm ^{31}P surface coil. To ensure equivalent excitation between methods, all studies were performed using adiabatic pulses. Mitochondrial function of the tibialis anterior muscle was assessed in the following order:

$P_i \rightarrow$ ATP flux Resting muscle ATP production (V_{rest}) was measured by determining the unidirectional $P_i \rightarrow$ ATP flux using the saturation-transfer (ST) technique. ^{31}P spectra were acquired with frequency-selective saturation of the γATP peak or with saturation at a downfield frequency equidistant from P_i. The T_1 of P_i under conditions of γATP saturation was measured using a 7-point inversion-recovery calibration. Fully-relaxed ^{31}P spectra (T_R = 35s) were obtained to determine metabolite concentrations in vivo.

Ischemic PCr decline Resting muscle ATP consumption during ischemia (V_{Isch}) was estimated from the rate of decrease of PCr during a 15min occlusion of the leg vasculature, accomplished with a blood pressure cuff inflated to 240 mmHg. V_{Isch} was derived from a linear fit of the decline in PCr during the ischemic period

PCr recovery Following 15min recovery from the ischemic protocol, oxidative capacity (V_{max}) was determined from the rate of PCr recovery after a 16-s maximal voluntary contraction (MVC) of the dorsiflexor muscles. Spectra were acquired with 2-s time resolution. PCr recovery for each subject was fitted to a monoexponential function. The duration of this contraction protocol gives rise to ~50% PCr depletion without a decrease in intracellular pH, which can modulate rates of PCr resynthesis.

Results

Representative spectra from the tibialis anterior muscle during a ST study are shown in Figure 1. The mean (± SE) change in PCr during the 16-s MVC protocol is shown in Figure 2. Summary data (mean, range) for each method are given in Table 1. Despite a 2-3 fold range in each of the measures of mitochondrial function, there were no significant associations between any of these measures (Pearson $r < 0.47$ for all analyses).

Figure 1

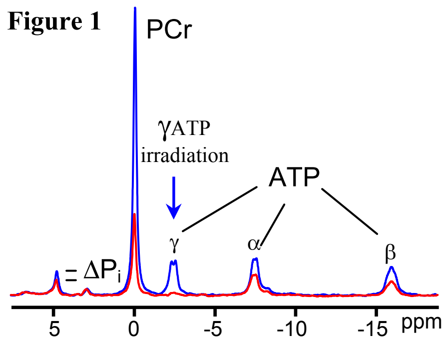

Figure 2

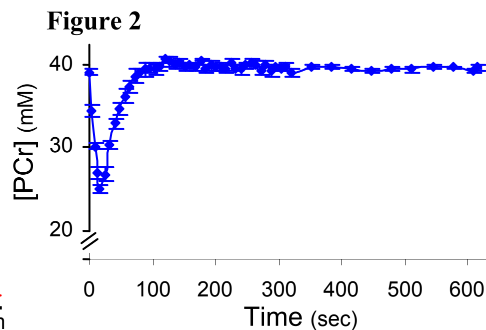

Table 1

ATP flux (mM/min)	Avg ± SE	Range
V_{rest}	9.05 ± 0.76	6.16 – 11.53
V_{Isch}	0.43 ± 0.07	0.21 – 0.71
V_{max}	58.33 ± 6.76	30.80 – 82.71

Conclusion

The results reveal a lack of correlation between these ^{31}P-MRS-based estimates of mitochondrial metabolism, even when measured within a single muscle in healthy individuals. Therefore these techniques apparently reflect independent parameters of oxidative function. Additional studies are needed to determine the application and interpretation of each methodology in the investigation of mitochondrial function in vivo.

Acknowledgements

This work was supported by NIH grants NIH/NIA K02 AG023582 (JKB) and NCCR/CTSA UL1 RR024139 (DEB).