Mitochondrial ultrasensitivity to ADP explains muscle energetics during recovery from exercise

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Introduction

The recent discovery of extensive and dynamic protein phosphorylation in the mitochondrial matrix subject to changes in extramitochondrial calcium concentrations (1) has rekindled the debate on the mechanistic underpinnings of energy balance in the active mammalian cell. An unresolved issue is whether these posttranslational modifications impact mitochondrial capacity for ATP synthesis or rather mitochondrial sensitivity to regulatory feedback signals of cellular ATP turnover rate. We have previously reported evidence that the latter aspect may well be central to cellular energy balance (2). Here, we report new evidence in the form of rich ^{31}P NMR spectroscopy data sets of ATP metabolism in human skeletal muscle and quantitative analysis of the post-exercise recovery state confirming our hypothesis that mitochondria are ultrasensitive (3) to variations in the cytosolic concentration of the ATP hydrolysis product ADP.

Materials & Methods

Six healthy subjects participated in the study (4 male, 2 female, age: 30 ± 12 years). ^{31}P MRS was performed at 1.5 Tesla (Gyroscan S15/ACS, Philips Medical Systems, Best, NL) using a 6-cm diameter surface coil placed over the *M. vastus lateralis*. Spectra were acquired using a repetition time of 3 s and 2 scans per spectrum (6 s time resolution) during a rest-exercise-recovery protocol. Exercise consisted of single-leg extensions every 1.5 s against incremental workloads for various durations (4).

PCr, inorganic phosphate (P_i) and ATP signals were quantified by time domain fitting employing the AMARES algorithm in the jMRUI software. Absolute concentrations were calculated after correction for partial saturation and assuming that [ATP] is 8.2 mM at rest. Intracellular pH was calculated from the chemical shift difference between the P_i and PCr resonances. The average end-exercise pH was 6.83 with an average standard deviation of 0.11 (8-12 data sets per subject). Cytosolic ADP concentration ([ADP]) was calculated from pH and [PCr] using a creatine kinase equilibrium constant of 1.66×10^9 M^{-1} and assuming that 15% of the total creatine is unphosphorylated at rest (4). The molar free energy of cytosolic ATP hydrolysis was calculated according to $\Delta G_p = \Delta G_p^{0'} + RT\ln([ADP][P_i]/[ATP])$, assuming $\Delta G_p^{0'}$ is -31.8 kJ/mol (2). The PCr resynthesis rate at time t ($V_{PCr}(t)$) was calculated from the derivative of the fitted PCr recovery time course $PCr(t)$ (Eqn. [1]):

$$PCr(t) = PCr_e - (\Delta PCr)\cdot\exp(-t/\tau_{PCr}) \qquad [1]$$

Covariations of this rate $V_{PCr}(t)$ reflecting mitochondrial oxidative phosphorylation flux with thermodynamic (ΔG_p) and kinetic ([ADP]) adenine nucleotide concentration functions were analyzed by nonlinear curve fitting of a modified (sigmoidal) Hill function of the form (Eqn. [2]):

$$V_{PCr}(t) = (Q_{max} - Q_{min})\cdot(x(t)/x_{0.5})^{n_H}/(1+(x(t)/x_{0.5})^{n_H}) + Q_{min} \qquad [2]$$

with n_H the Hill coefficient, $x_{0.5}$ the x-value at half-maximal V_{PCr}, and Q_{max} and Q_{min} the maximal and minimal net fluxes (2).

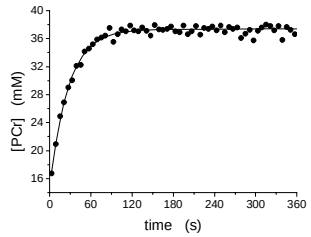

Figure 1. PCr recovery curve for one subject. A mono-exponential function (dark line) was fit to the actual data (filled circles). The time constant for PCr recovery was 26.9 s.

Results

Figure 1 shows the raw data and mono-exponential fit of the PCr recovery from one data set. The function $V_{PCr}(t)$ determined from such fits was next correlated with ΔG_p at each measurement time point to construct the thermodynamic flow-force relation of mitochondrial oxidative phosphorylation (**Figure 2**). This relation was characterized by unconstrained fitting of Eqn. [2] to the data (Figure 2, solid line). Almost the full range of sustainable energy balance states was covered by the experimental data points, resulting in accurate estimation of the flux asymptotes Q_{max} and Q_{min} (0.76 and -0.02 mM/s, respectively, for this particular subject). In the final step of the analysis, these constraints for Q_{max} and Q_{min} were imposed on the fit of Eqn. [2] to the ([ADP], V_{PCr}) data (**Figure 3**, solid line) to determine the affinity and apparent kinetic order of mitochondrial ADP sensing. The results of the analysis for each of the subjects studied are summarized in **Table 1**. Group mean values for $[ADP]_{0.5}$ and n_H were 22.0 ± 2.9 μM and 1.86 ± 0.15, respectively.

Discussion & Conclusion

In a previous investigation of the apparent kinetic order of the transduction function of mitochondrial ADP sensing, we analyzed covariations of the mitochondrial ATP synthesis rate with cytosolic ΔG_p and [ADP] in contracting muscle measured typically at six electrical stimulation frequencies controlling contractions in each subject (2). Only by pooling the data of six subjects, adequate sampling of the underlying physiological relationship was achieved to determine the Hill coefficient. Here, this problem was solved by taking an alternative approach – i.e, by reconstructing the relationship between mitochondrial ATP synthesis flux and ΔG_p and [ADP], respectively, from the densely sampled PCr recovery time course *following* muscle contractions (Figures 2 and 3). This allowed for accurate estimation of the mitochondrial flux asymptotes and thereby the Hill coefficient of the ADP transduction function in individual subjects (Table 1). In all six subjects studied, n_H was 2. This result confirms our hypothesis that mitochondria are ultrasensitive to the feedback signal of variations in cytosolic ATP turnover rate, [ADP] (2).

References: 1. Hopper et al. *Biochemistry* 45, 2524-2536 (2006); 2. Jeneson et al. *J Biol Chem* 271, 27995-27998 (1996); 3. Koshland et al. *Science* 217, 220-225 (1982); 4. van den Broek et al. *Am J Physiol (Cell Physiol)* 293, C228-C237 (2007).

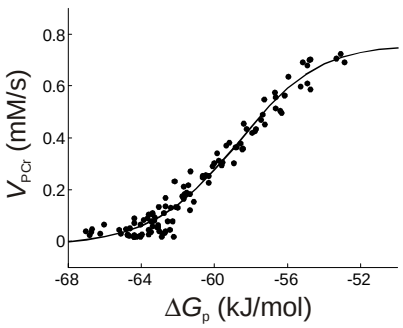

Figure 2. PCr resynthesis rate as a function of the molar free energy of cytosolic ATP hydrolysis (filled circles) for subject 2. The unconstrained, 4-parameter fit of Eqn. [2] to the data is shown by the solid line.

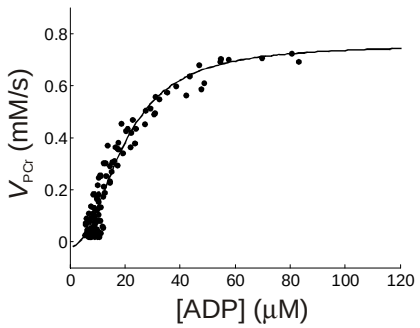

Figure 3. PCr resynthesis rate as a function of ADP concentration (filled circles) for subject 2. Eqn. [2] was fitted to the data, with Q_{max} and Q_{min} constrained to the values from Figure 2 (solid line).

Table 1. Results of curve fitting of Eqn. [2] to the ([ADP], V_{PCr}) data for the 6 subjects. Q_{max} and Q_{min} were constrained to values obtained from the flow-force relation.

subject	$[ADP]_{0.5}$ (μM)	n_H
1	25.0	1.91
2	19.4	2.13
3	17.6	1.71
4	23.5	1.86
5	24.2	1.79
6	22.3	1.73