Kinetic model-based analysis of dynamic 31P MRS data on ATP metabolism in rat hindlimb muscle

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Introduction.

Dynamic ^{31}P NMR spectroscopy (^{31}P MRS) has longtime been used to probe the homeostatic performance of the integrated biochemical networks involved in cellular energy balance in a variety of human diseases including heart failure and diabetes [1]. Physiological information extraction from this type of study has in most cases, however, been limited to macroscopic scoring of mitochondrial function, typically on basis of curve-fitting fitting of phosphocreatine (PCr) recovery dynamics [1]. Recent advances in computational modeling of these biochemical networks now offer a tool to link these macroscopic ^{31}P MRS observations to the rich knowledge base on the molecular components interacting in these networks [2]. Here, we explored the potential of such kinetic model-based analysis of dynamic ^{31}P MRS recordings of ATP metabolism in muscle to extract information on the activity of a key mitochondrial enzyme complex, *Pyruvate Dehydrogenase* (PDH). This particular molecular component of the mitochondrial metabolic network was chosen for its potential relevance in the pathophysiology of diabetes.

Materials & Methods.

^{31}P Magnetic Resonance Spectroscopy. Measurements were conducted in Swiss-Webster rats using a horizontal bore 4.7 Tesla Bruker MR spectrometer. A lower limb of the animal was positioned over a 12 mm diameter three turn double tuned ^{1}H/^{31}P surface coil and its Achilles tendon was ligated and fixed to a home-built strain gauge for mechanical recordings during muscle contractions. ^{31}P NMR spectra were recorded continuously during contractions and metabolic recovery (block excitation pulse; TR 1.4 s; SW 4000 Hz; dwell time 66 ms; 4 FIDs). Twitch contractions of the lower hindlimb muscles were elicited by electrical stimulation of the sciatic nerve at frequencies ranging between 0.25 and 2.0 Hz in random order. Each frequency was maintained for 300 s with 600 s time rest intervals in between bouts.

Data processing. PCr, inorganic phosphate (P_i) and ATP resonances were fitted in the time domain using the AMARES algorithm in the jMRUI software (www.jMRUI.org). Absolute concentrations were calculated after correction for partial saturation. Adenine nucleotide and creatine poolsizes were measured by HPLC [3]. Intracellular pH was calculated from the P_i and PCr resonance frequencies; ADP was estimated from the creatine kinase equilibrium [4]. ATP turnover at each contraction frequency rate was determined from the initial rate of PCr consumption [4]. Mechanical force recordings were used to correct for any changes in this rate associated with onset of muscle fatigue. Mitochondrial ATP synthesis rate was determined from the PCr and muscle pH timecourses as described elsewhere [4].

Computational Modeling. A kinetic model of cellular ATP metabolism integrated with detailed mitochondrial bioenergetics (including kinetic models of the TCA cycle, electron transport system and oxidative phosphorylation, as well as substrate and cation transport systems across the mitochondrial inner membrane [5]), was used as computational platform for numerical analysis of the experimental data and hypothesis testing. Three alternative hypotheses for PDH activation during exercise were tested: **(1.)** PDH activity is maintained at basal level; **(2.)** PDH activity switches to maximal activity upon muscle contraction; **(3.)** PDH activity is variable, depending on the duty cycle of contraction. For each hypothesis, the PDH kinetic model was adapted correspondingly (e.g. for hypothesis *3*, time constants of activation at onset of stimulation and deactivation at onset of recovery, respectively, were introduced) and parameterized by fitting to experimental datasets (*learning* set). Independent experimental datasets (*test* set) were used for hypothesis testing.

Results.

Figure 1 shows a typical example of the rich ^{31}P MRS quantitative information on the dynamics of bulk PCr and Pi concentrations in posterior rat hindlimb muscles during a series of three consecutive contraction-recovery bouts at different contraction frequencies (single experiment). Figure 2 shows model simulations of the PCr dynamics for these three contraction frequencies against experimental data for three alternative hypotheses on the kinetic regulation of PDH activity in muscle. These and other 31P MRS recordings showed that the model of constitutive basal PDH activity (*hypothesis 1*) was incompatible with the data. The converse model of constitutive maximal activation of PDH at any duty cycle of contraction (*hypothesis 2*) resulted in structural underestimation of the magnitude of PCr depletion during contraction. A satisfactory match between model predictions and experimental data was found for variable PDH activity (*hypothesis 3*).

Discussion.

A prerequisite for any merit to the type of investigation presented here is the availability of a robustly parameterized kinetic model that passed multiple rounds of invalidation testing. Significant progress towards achieving this standard has been made in kinetic modeling of mitochondrial oxidative metabolism [2]. This study presents a first step in our efforts to develop kinetic model-based analysis of dynamic ^{31}P MRS data into a mature investigative tool to probe into mitochondrial physiology beyond the macroscopic scale, and test hypotheses on the molecular basis of energy balance in mammalian tissues. Currently, independent data are being collected to test the validity of hypothesis *3*. The end objective is to apply this tool in the clinical investigation of failing ATP homeostasis in human disease.

References.

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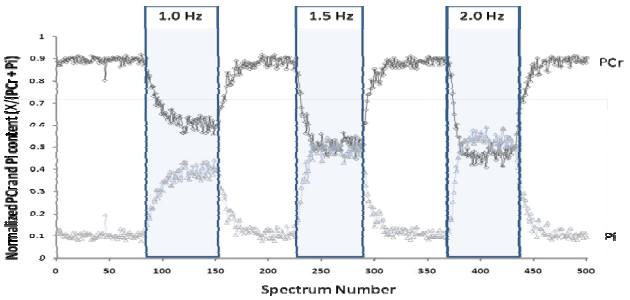

Figure 1. **Dynamics of PCr and Pi during rest-stimulation-recovery bouts in a single experimental run.** Muscles were paced electrically by electrodes placed upon the sciatic nerve and stimulated at 1, 1.5 and 2 Hz (shaded boxes). Each data point represents information from 1 spectrum (8 sec). PCr and Pi were normalized to their content (X/(PCr+Pi). Note that the changes in PCr and Pi are reciprocal and stoichiometric. The frequencies were chosen to induce sustainable bioenergetic steady-states below maximal mitochondrial capacity.

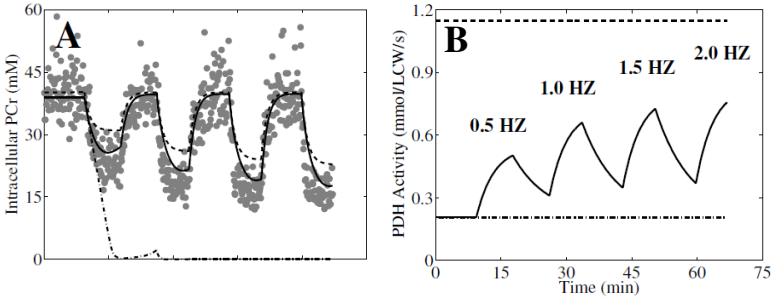

Figure 2. **Model simulations versus XP data of rest-stimulation-recovery metabolic cycles in a single run.** (A) Comparison of model simulations to experimental data on PCr dynamics during stimulations and recovery. Model simulations for three different hypotheses on PDH activation during exercise are shown various lines. *Hypothesis 1*: dashed-dotted lines; *Hypothesis 2*: dashed lines; *Hypothesis 3*: solid lines. (B) model prediction of corresponding PDH activity over the course of the experimental protocol.