

Exercise ability is determined by muscle ATP buffer content, not Pi or pH

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Introduction

Skeletal muscle fails to maintain power output during intense work, a property commonly known as muscle fatigue. In the debate of its molecular basis, the field has recently witnessed a paradigm shift. Intracellular accumulation of inorganic phosphate (P_i), has replaced muscle acidification as main culprit (1). Specifically, formation of calcium phosphate (CaP_i) complex in the sarcoplasmic reticular (SR) calcium stores as a result of intracellular accumulation of this ATP hydrolysis product during strenuous work is a leading hypothesis (1). Its significance *in vivo* has, however, not been tested. Here, we investigated this hypothesis in human subjects *in vivo*. The dissociation between maximal energetic depletion and maximal muscle acidification that is particular to two-legged voluntary exercise (2) was exploited to design an experiment in which the muscle concentrations of P_i and H^+ at the onset of bicycling exercise were manipulated quasi-independently.

Materials & Methods

Ergometry. An MR-compatible bicycle ergometer constructed from non-ferrous components (3) was used in all studies. Six healthy male subjects (mean age 29 years) twice performed three consecutive bouts of supine bicycling against supramaximal load set in each subject to cause exhaustion at 60 ± 5 s. During bout I subjects exercised until exhaustion while during bouts II and III, subjects exercised for 20s. The bouts were separated by 30s rest during which muscle phosphocreatine (PCr) and P_i content were allowed to partly recover while muscle pH continued to drop. Subjects were instructed to maintain a pedaling frequency of 80 rpm.

Set 1 was performed inside a 1.5 T Philips S15/ACS scanner. Set 2 (HDsEMG) was performed outside the magnet. One week of rest was observed between studies. **³¹P MRS.** ³¹P spectra were acquired from the medial head of the quadriceps muscle of the right leg using a 6 cm surface coil. During exercise RF pulsing was synchronized with cyclic extension of the right leg (3). Spectra were acquired using an AHP pulse with a TR of 3 s; 2 FIDs were summed per spectrum yielding 6 s time resolution. PCr, inorganic phosphate (P_i) and ATP resonances were fitted in the time domain using the AMARES algorithm in the jMRUI software package. Absolute concentrations were calculated after correction for partial saturation and assuming adenine nucleotide and creatine poolsizes of 8.2 and 42.7 mM, respectively (2). Intracellular pH was calculated from the chemical shift difference between the P_i and PCr resonances (2).

HDsEMG. Multi-channel (10x13) surface EMG recordings from the medial head of the quadriceps muscle of the right leg were obtained as described elsewhere (4). The data were next digitally band-pass filtered (10–500 Hz). The EMG amplitude, expressed by the root mean square (RMS) was calculated from the monopolar data. The RMS was calculated over the active period of every cycle (0.35s corresponding to 718 data points) for every channel individually and averaged afterward.

Results

Figure 1 shows a typical ³¹P NMR spectrum of the quadriceps muscle acquired at the end of 30 s rest after completion of exercise bout I – i.e. immediately before the start of exercise bout II. Clearly, intracellular P_i was still significantly elevated compared to resting muscle and the intracellular milieu was significantly acidified (pH 6.5) when the subject resumed bicycling against supramaximal load at a pedaling rate 80 rpm. **Figure 2** shows the timecourses of the intramuscular concentration of PCr (A), P_i (B) and pH (C) of an individual subject during a set of serial exercise-recovery bouts. In this particular subject, [PCr] had dropped to 5 mM at exhaustion. During metabolic recovery between bouts I and II, and II and III, [P_i] decreased to 20 mM and pH dropped to 6.5-6.6. **Figure 3A** shows the mean amplitude (RMS; in mV) of the EMG signals picked up from the medial head of the quadriceps muscle by the array of electrodes during each bout of bicycling against supramaximal load (single individual). **Figure 3B** shows a plot of the pedaling frequency recorded during the exercise bouts versus the intramuscular P_i and H^+ concentrations at that time.

Discussion

This study has demonstrated that healthy human subjects are able to initiate and shortly sustain bicycling exercise against supramaximal load maintaining a pedaling frequency of 80 rpm by recruiting the same motor units in the quadriceps muscle of the upper leg, independent of the intramuscular P_i concentration or pH at the onset of exercise. In all subjects, however, failure to maintain this high pedaling frequency coincided with 85% depletion of the PCr, the main ATP buffer in muscle. These findings suggest a limited role of CaP_i complex formation or H^+ myofilament desensitization in muscle fatigue *in vivo*. Instead, they point to a major role for local ATP depletion at key nodes in the excitation-contraction coupling network as a result of ATP buffer depletion.

References

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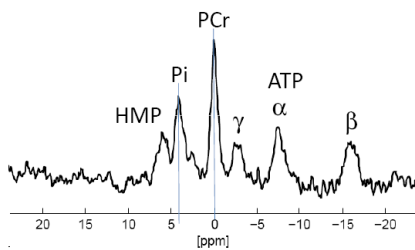


Figure 1. ³¹P NMR spectrum of the quadriceps muscle of a subject obtained at the end of 30 s recovery after bout I – i.e., the starting point of exercise bout II. HMP, hexose monophosphates.

Figure 2. Timecourse of PCr concentration (A), P_i (B) and pH (C) in the quadriceps muscle of an individual subject during one complete set of 3 exercise-recovery bouts (0-180 s excerpt). Shaded blocks indicate exercise bouts I, II and III.

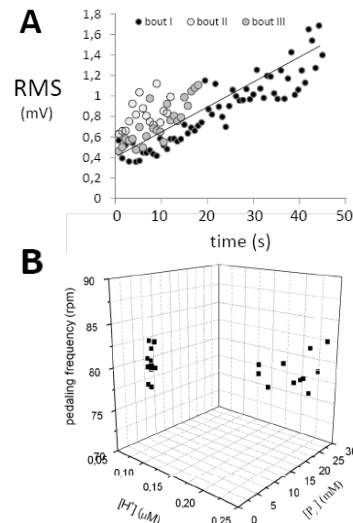
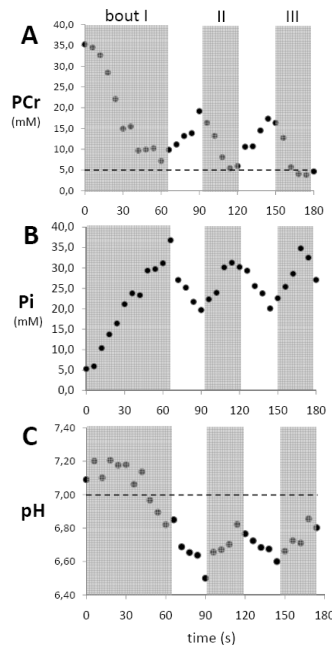


Figure 3. (A) amplitude (mV) of the EMG recordings of the quadriceps muscle during exercise bouts I, II and III (same subject as Figure 2) (B) pedaling frequency (rpm) at various intramuscular concentrations of P_i and H^+ during supine bicycling against supramaximal load.