In-magnet bicycling exercise: a novel 31P MRS window on the energetics of human locomotion

J. A. Jeneson[1], J. P. Schmitz[1,2], and K. Nicolay[1]

[1]Biomedical NMR, Department of Biomedical Engineering, Eindhoven University of Technology, Eindhoven, Netherlands, [2]BioModeling and Bioinformatics Group, Department of Biomedical Engineering, Eindhoven University of Technology, Eindhoven, Netherlands

Introduction

The clinical standard test of patient fitness is the upright bicycle exercise test. For various reasons, no proper equivalent human MR exercise test has been available. Past designs of exercise protocols in clinical MR studies typically involved single limbs (i.e., arm or leg) (1). While informative, these designs generally failed to put any significant demands on the patient cardiovascular system. Indeed, a vast majority of studies involving single limb exercise have been plagued by unsolicited muscle acidification impairing straightforward clinical interpretation of post-exercise phosphocreatine (PCr) recovery kinetics (2,3). We previously reported superior robustness of muscle energy and proton balance during two-legged exercise (4). The particular ergometer used in that study, however, had a number of shortcomings including low cycling rates and high mechanical inertia that rendered it unfit for use in clinical studies. Here, we report on ^{31}P MRS studies employing a novel ergometer that for the first time offers true in-magnet bicycling exercise testing of human subjects and is highly suited for clinical investigation.

Materials & Methods

Ergometer. A bicycle ergometer was constructed from non-ferrous components. The mechanical load was controlled by adjusting the brake force exerted on a wooden flywheel. The position of the pedals was registered using an optic fiber to synchronize RF pulsing with extension of the right leg during bicycling. A metronome was used to set the pedaling frequency (typically 80 rpm).

^{1}H and ^{31}P MRS. Nine physically fit male subjects participated in the study (mean age 25 years). Studies were performed on a 1.5-Tesla whole-body scanner (Gyroscan S15/ACS, Philips Medical Systems, Best, NL). The magnet was shimmed using a rectangular ^{1}H surface coil (typical H_2O linewidth 25 Hz) after which a 6-cm diameter ^{31}P surface coil was fastened over the medial head of the quadriceps muscle of the right leg. ^{31}P spectra were acquired using an AHP pulse. A first set of fully relaxed spectra (TR 15 s) was acquired from resting muscle. Subsequent spectra were acquired with a TR of 3 s. During exercise, this was achieved by setting the spectrometer gating delay to 2.5 s. RF pulsing was controlled by and recorded on a PC using dedicated software written in Matlab (). 4 FIDs were summed per spectrum yielding 12 s time resolution. Subjects performed rest-exercise-recovery protocols at various workloads including maximal. Each workload was maintained for 180 s.

Data processing. PCr, inorganic phosphate (P_i), hexose monophosphate (HMP) 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 (4). Intracellular pH was calculated from the chemical shift difference between the P_i and PCr resonances (4).

Results

Figure 1 shows a typical set of ^{31}P NMR spectra acquired during a rest-exercise-recovery experiment. The usual stoichiometric changes in PCr and Pi at constant ATP were observed as well as significant dynamics of HMP. **Figure 2** shows the summed ^{31}P NMR spectrum of 3 FIDs collected over the final 36 s of 180 s bicycling at maximal sustainable workload. Heart rate directly following cessation of exercise was 150 $\pm$ 15 bpm. PCr declined to 10% of initial indicating that this workload recruited all motor units in the quadriceps muscle. Intracellular pH in all sampled fibers was 6.8. **Figure 3** shows the mean [PCr] and [HMP] dynamics ($\pm$ SD) during exercise against three incremental workloads (including maximal) and recovery for 9 subjects. [PCr] declined to 12.3 $\pm$ 2.4 mM concomitant with intracellular accumulation of HMP of up to 5.7 $\pm$ 1.7 mM. Lowest end exercise pH was 6.8. During recovery, the initial rate of PCr resynthesis was 0.7 $\pm$ 0.3 mM/s. This rate did not exceed rates measured following exercise at lower workloads (data not shown) indicating maximal mitochondrial ATP synthesis flux was experimentally determined. The HMP concentrations peaked 60 s into recovery at 6.9 $\pm$ 1.3 mM and returned to resting levels at the surprisingly slow rate of 8.0 $\pm$ 0.5 μM/s.

Discussion

We have developed and successfully implemented hardware on a clinical 1.5T MR scanner for in-magnet bicycling exercise testing of human subjects. Three features of the first study in healthy subjects reported here are of particular interest to the (clinical) investigation of human exercise performance. Firstly, ATP metabolism in quadriceps muscle was studied over a 100-fold dynamic range of ATP turnover with little intramuscular acidification (Figures 1 and 2). Secondly, the cardiovascular and pulmonary system were significantly challenged during the exercise, with heart rates going up as high as 150 bpm. Thirdly, it provides a ^{31}P MRS window on glycogen metabolism through the dynamics of hexose monophosphate resonances during exercise and recovery (5) (Figure 3). As such, the setup for in-magnet bicycling exercise that we developed offers a powerful novel MR-based investigative tool in the clinical investigation of human disease in which exercise intolerance is a prominent symptom and complication (e.g. heart failure and type 2 diabetes).

References

1. Prompers et al (2006) *NMR Biomed* 19: 927-53; **2.** Bendahan et al (1990) *FEBS Lett* 272: 155-58; **3.** van den Broek et al (2007) *Am J Physiol* 293: C228-37; **4.** Jeneson and Bruggeman (2004) *FASEB J* 18:1010-12; **5.** Walter et al. (1999) *J Physiol* 519: 901-10.

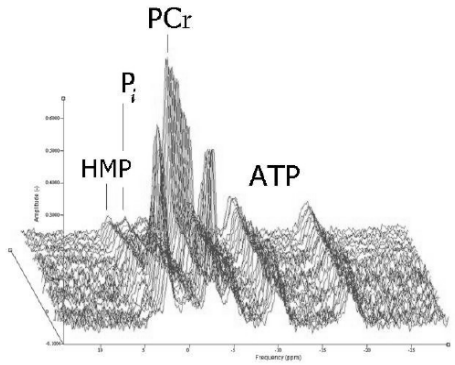

Figure 1. 31P NMR spectra from the medial head of the quadriceps muscle of a subject obtained during bicycling exercise at 80 rpm against high braking resistance and subsequent recovery (36 s time resolution; 3 summed FIDs per spectrum). HMP corresponds to hexose monophosphates,.

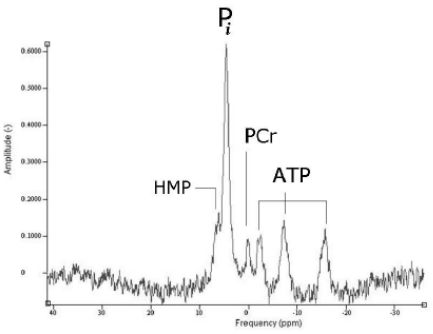

Figure 2. ^{31}P NMR spectrum from the medial head of the quadriceps muscle of a subject performing bicycling exercise at the maximal workload sustained for 180 s. The lineshape of the Pi resonance was highly symmetrical indicating homogeneous pH across the sampled muscle mass. Intracellular pH was 6.8.

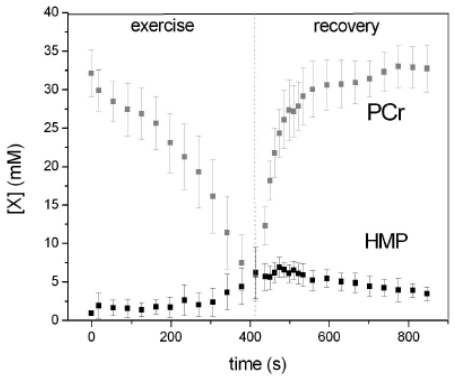

Figure 3. Mean [PCr] and [HMP]dynamics ($\pm$ SD; n=9 subjects) during bicycling exercise against three incremental workloads including maximal, and recovery. Lowest end-exercise pH was 6.8. Analysis of [PCr] recovery timecourse by nonlinear curve fitting (4) revealed two kinetic components (timeconstants: 16 $\pm$ 2 and 68 $\pm$ 5 s, respectively).