

³¹P MRS reveals post-exercise impacts on energy metabolism of heart failure rats

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PURPOSE: Exercise improves the cardiac performance of patients with myocardial infarction (MI), however, the relative beneficial and/or detrimental effect of moderate versus high intensity training is uncertain. Phosphorus (³¹P) magnetic resonance spectroscopy (MRS) allows detection of high energy phosphate in the heart muscles; adenosine triphosphate (ATP) and phosphocreatine (PCr). ATP is the direct energy source for all energy consuming reactions in the heart and PCr is the major compound for energy storage and transport¹. Reduced myocardial PCr/ATP ratio in heart failure patients correlates with the New York Heart Association (NYHA) classes². A clinical study showed that PCr/ATP ratio might be a stronger predictor for cardiovascular mortality than functional or clinical indexes³. The purpose of this study was to investigate myocardial phosphate energy reservoir in the left ventricle from rats with MI after exposure to moderate intensity (Mod) and high intensity (High) aerobic interval training regimens.

METHODS: Animal models: Rats were randomized to either sham or MI operations. MI was induced by ligation of the descending artery, as previously described⁴. After 4 weeks, rats with MI operation were examined by echocardiography to determine the extent of MI. Only rats with MI between 40-50% of the left ventricle were included to this study. Exercise training: High intensity training was performed as uphill running (25°), alternating between 4 min at 85%-90% of maximal oxygen uptake (VO_{2max}) and 2 min at 50% of VO_{2max} for 60 min/day, 5 days/week, for 8 weeks. Mod-groups ran uphill (25°) alternating between 4 min at 65-70% of VO_{2max} and 2 min at 50% of VO_{2max} for the same distance as High-groups (isocaloric). Mod-groups started to exercise for 80 min and ended up at performing 110 min after 8 weeks of exercise training to match the amount of work performed by High-groups. The experiments were performed according to the Guide for the Care and Use of Laboratory Animals (National Institutes of Health Publication No. 85-23, revised 1996). Tissue extraction: The rats were anesthetized and the hearts were removed and placed in ice-cold saline for dissection. To avoid fibrotic and ischemic parts, tissue from the septum towards the apex was cut out from every heart and immediately frozen. Time from removal of the heart to snap freeze was approximately 1 min and did not differ between groups. MR experiments: Snap-frozen myocardial tissue samples were extracted using perchloric acid as described in detail previously⁴. NMR experiments were performed on a 14.1 T Bruker spectrometer (Bruker Avance III 600 MHz/54 mm US-Plus) equipped with a multinuclear QCI CryoProbe (Bruker BioSpin, Ettlingen, Germany). High resolution ³¹P MR spectra were obtained with proton decoupling, a 30° flip angle, 8192 scans, TR=3.62 s, spectral width of 14577 Hz into 47104 data points. MR spectra were analyzed using jMRUI software⁵ and the metabolites signals fitted with the AMARES method⁶. Metabolites were quantified by normalizing the area under the peak to the total area under the metabolites; inorganic (Pi), ATP, and PCr. In situ mitochondrial respiration: Mitochondrial function was assessed using high-resolution respirometry (using oxygraph-2k, Oroboros instruments, Austria) in chemically permeabilized heart muscle fibers. Saturating concentrations of combined malate, glutamate (supplying electrons to complex I) and succinate (supplying electrons to complex II) results in maximal oxidative phosphorylation, expressed as pmol O₂-s⁻¹-mg⁻¹ wet weight. Statistics: GraphPad Prism (GraphPad Software, Inc. V 4.03, CA, USA) was used to compare the mean difference between metabolites concentrations across the exercised groups using the non-parametric Mann-Whitney test. *P*-value < 0.05 was considered statistically significant.

RESULTS: High intensity training increased the oxygen consumption by 40% while the moderate intensity training increased the maximal oxygen consumption by 20%. Both modes of exercise training increased ATP concentration in sham and MI groups (Fig. 1). PCr levels in MI group were depleted significantly compared with sham control (*P* < 0.05), although a trend toward increased PCr storage was observed in the post-exercise MI group. Maximal oxidative phosphorylation of mitochondria was reduced in MI heart compared to sham (162 vs. 243 [pmol/s.mg], respectively). Exercise did not increase the maximal respiration (176 and 181 [pmol/s.mg], respectively in moderate- and high intensity training).

CONCLUSION: ATP production increased with training but seems not to be consumed to produce PCr, evidenced by the lower PCr levels in MI rats (whether they trained or not). The results demonstrate the potential of ³¹P MRS to investigate the efficacy of different exercise regimens to improve cardiac performance. When translated to the clinic, this technique may benefit patients with MI heart by determining the effect of training by monitoring the high energy metabolism in vivo.

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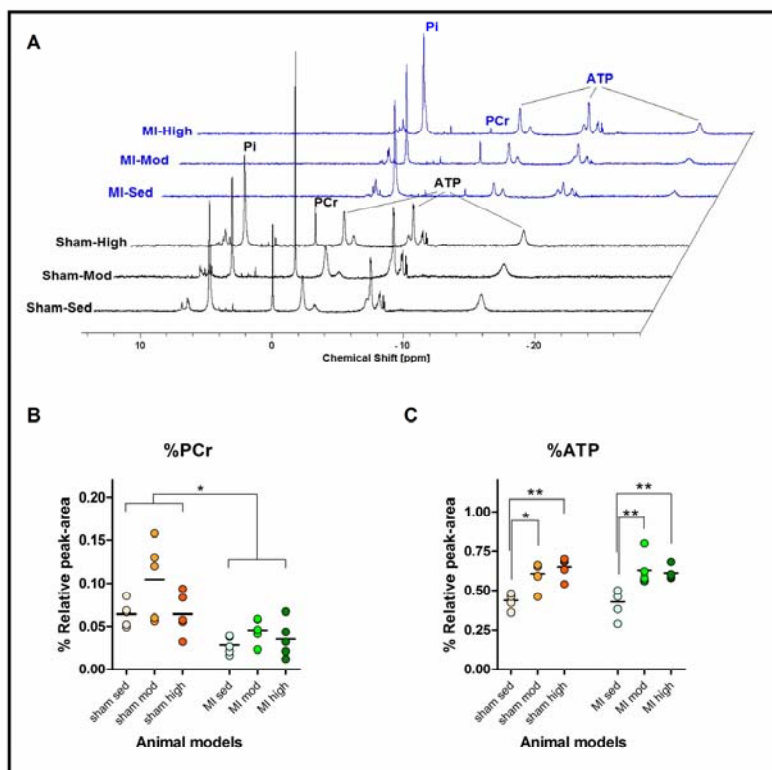


Figure 1: (A) Examples of high resolution ³¹P NMR spectra of heart muscle tissue extracts obtained from MI and healthy rat models; sedate, moderate- and high-intensive trained. (B) MI caused significant decrease in PCr levels compared with the sham control group. Dots indicate the normalized PCr levels measured from individual MR spectra. (C) ATP production increased post-training. Symbols * and ** denote *P* < 0.05 and *P* < 0.01 respectively.