METHODS: Animal models: Rats were randomized to either sham or MI operations. MI was induced by ligation of the descending artery, as previously described. 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 in this study. Exercise training: High intensity training was performed as uphill running (25°), alternating between 4 min at 85%-90% of maximal oxygen uptake (VO2max) and 2 min at 50% of VO2max for 60 min/day, 5 days/week, for 8 weeks. Mod-groups ran uphill (25°) alternating between 4 min at 65-70% of VO2max and 2 min at 50% of VO2max, 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 previously. 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 90° flip angle, 8192 scans, TR=3.62 s, spectral width of 14577 Hz into 47104 data points. MR spectra were analyzed using jMRUI software and the metabolites signals fitted with the AMARES method. Metabolites were quantified by normalizing the signals to the creatine signal. 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 the sham control 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.