Phosphorus MRS study of a murine model of peripheral arterial disease

Vladimír Mlynárik[1], Maxime Pellegrin[2], Cristina Cudalbu[1], Lucia Mazzolai[2], and Rolf Gruetter[1,3]
[1]*Laboratory of Functional and Metabolic Imaging, EPFL, Lausanne, Switzerland,* [2]*Service of Angiology, University Hospital of Lausanne, Lausanne, Switzerland,*
[3]*Departments of Radiology, Universities of Lausanne and Geneva, Switzerland*

Introduction: Peripheral arterial disease (PAD) consists of stenosis/obstruction of the lower limb arteries leading to reduced vascular perfusion and ischemia. Ischemia is thought to initiate a series of dynamic and natural events, such as angiogenesis, arteriogenesis, or enhanced muscle metabolic capacity seeking to restore tissue perfusion. PAD is mainly due to atherosclerosis and is associated with increased cardiovascular morbidity and mortality. Along with lifestyle changes, the treatment of PAD involves exercise therapy. However, precise mechanisms by which exercise provides clinical benefit are still unknown.

^{31}P localized MR spectroscopy can be useful for monitoring PAD since a decrease of PCr is the earliest measurable intracellular event in energy metabolism. PCr regeneration occurs exclusively within the mitochondria, and, because this depends entirely on the cell's capacity for oxidative phosphorylation and oxygen supply, it represents an ideal parameter for detecting metabolic evidence of tissue ischemia. In our murine hind-limb ischemia model, we used phosphorus localized MR spectroscopy for studying effect of treadmill exercise on PAD.

Experimental: Unilateral chronic ischemia was induced in 14 to 16-week old hypercholesterolemic ApoE$^{-/-}$ male mice (IFFA CREDO, L'Arbresle Cedex, France) by ligating right common iliac artery. Twelve mice were anesthetized using isoflurane inhalation (1% to 2% in oxygen) and the common iliac artery was exposed and ligated with 7-0 silk suture proximal to the internal-external iliac junction. The iliac vein and nerve were preserved. The abdomen incision was then closed with resorbable 5-0 silk suture. One week after surgery Laser-Doppler blood perfusion measurements showed about 70% decrease of the blood perfusion in the ischemic leg. Six mice were exercised on a rodent motor treadmill (Columbus Instruments) starting 1 week after surgery. Each training session started at a speed of 9 m/min for 3 minutes with an increase of 2 m/min every 3 minutes until a maximum speed of 19 m/min was reached. The training was performed until mice were exhausted. The mean training time per day was 58 ± 12 min.

Animals were measured on a 9.4 T Varian VNMRS spectrometer (Varian, Palo Alto, CA USA) in the supine position. Body temperature was maintained at 37.5 ± 1.0 °C by circulating warm water. A home-built 18 mm-diameter dual ^{1}H quadrature/ 10 mm-diameter ^{31}P single-loop surface radiofrequency coil was used as a transceiver. For the localization, T2–weighted turbo-spin-echo images were obtained in the axial plane using a field of view 30 mm × 30 mm and 1 mm slice thickness. VOIs of about 60 mm^3 were chosen in femoral muscles of the hind limbs (Fig. 1). The static field homogeneity in the selected VOI was adjusted by the EPI version of FASTMAP using the ^{1}H signal of water (1). Spectroscopic localization was achieved by outer volume saturation, *i.e.*, by applying slice selective inversion in the upper horizontal plane and saturation pulses in all planes around the selected volume of interest (2). 160 transients were collected with a repetition time of 4 seconds. The total measurement time for imaging and ^{31}P spectroscopy of one leg was about 1 hour. The peak intensities of PCr and γ-ATP were obtained by fitting to a Lorentzian function using AMARES (3) from the jMrui software (http://sermn02.uab.cat/mrui/).

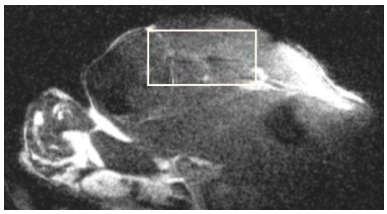

Fig. 1. Turbo-SE image of a mouse hind limb with the VOI selected for spectroscopy

Results: Ligating right common iliac artery led to a significant decrease in PCr/γ-ATP compared to sham-operated controls one week after surgery (Fig. 2). Interestingly, the PCr/γ-ATP ratio was reduced not only in the ischemic but also in the healthy leg (Fig. 3). No change in concentrations of inorganic phosphate or other phosphorus-containing metabolites was observed. Five weeks after operation the PCr/γ-ATP ratio increased in both ischemic and non-ischemic legs to levels slightly higher than those in sham-operated animals. There was no significant difference between sedentary and treadmill-exercised animals, although the PCr/γ-ATP ratio was slightly smaller in the ischemic limbs of sedentary mice (Fig. 3).

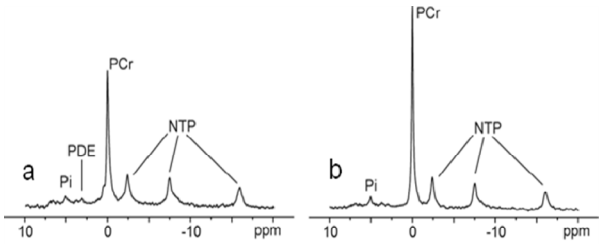

Fig. 2. ^{31}P spectrum of femoral muscles of an ischemic leg (**a**) 1 week after surgery and (**b**) 5 weeks after surgery

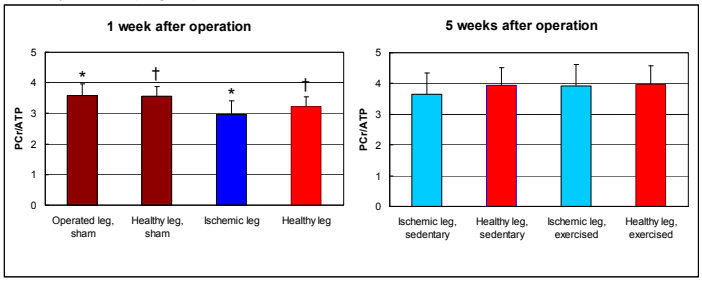

Fig. 3. Relative concentrations of PCr in femoral muscles of the ischemic and healthy legs 1 week and 5 weeks after operation. Pairs of symbols (*,†) denote significantly different values. All the values measured 5 weeks after operation were significantly different from those measured in the corresponding legs 1 week after surgery ($p < 0.05$).

Discussion: We showed that hind limb ischemia led to the decrease in PCr/γ-ATP in femoral muscles. The modification of metabolism was also seen in the muscles of the contralateral leg. Recovery of PCr/γ-ATP in the ischemic leg of the exercised mice was slightly faster than that of the sedentary mice, however, this difference was not statistically significant.

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