Simultaneous non-invasive determination of tissue perfusion, arterial blood pressure and peripheral vascular resistances in mice

J. Ménard[1], E. Giacomini[1], C. Baligand[1], D. Bertoldi[1], and P. Carlier[1]
[1]NMR laboratory AIM-CEA, Institute of Myology, Paris, France

Introduction: Vasodilatation reserve is a key parameter to assess organ adaptation to increased metabolic demand. We have previously shown that peripheral vascular resistances can be determined non-invasively in rats by combining quantification of skeletal muscle perfusion by ASL-NMRI techniques and systemic arterial blood pressure (BP) using dynamic NMR angiography (MRA)[1]. In this work, we automated the measurement process and adapted the NMR setup and sequences to mice. As a first example of application, we measured the vascular resistances in skeletal muscle of type-2 diabetic mice.

Materials and methods: Experiments were performed with a *Bruker Biospec* 4T NMR system equipped with a 20cm diameter 200mT.m^{-1} gradient insert. Ten week-old male diabetic (C57Bl6 db$^-$/db$^-$) and control (C57Bl6 db$^-$/db$^+$) mice (Janvier, France) were anaesthetized with 1.7% of isoflurane in medical air delivered at 1.5l.min^{-1} and placed in supine position on a heating pad. The experimental set up consisted in three ^{1}H coils proportioned to mouse: one birdcage transmitter and two surface receivers, with active decoupling.

NMR quantification of perfusion in murine skeletal muscle[2]: The ASL-NMR imaging sequence is a single slice RARE imaging (single shot, inter-echo spacing: 2.9ms, RARE factor: 32, in-plane resolution: 0.39x0.93mm^2) combined with a pulsed ASL module variant SATIR (SATuration Inversion Recovery): (Transit time (delay between tagging and imaging): 1.3s, TR (delay between two consecutive tagging modules): 9s).

NMR systolic and diastolic BP determination in mouse: Changes in the angiographic arterial signal of the caudal artery were monitored by MRA imaging during progressive tail cuff inflation:

- MRA imaging of the mouse tail was a standard fast gradient echo time-of-flight (TOF) sequence. (TR: 13ms, TE: 2.8ms, α: 55°, slice thickness: 3mm, in-plane resolution: 97x137um^2, image acquisition time: 2.5s).
- The custom-designed pneumatic device dedicated to tail cuff inflation was composed of a dynamic electro-pneumatic regulator (*ITV001, poweraire)* controlled with a custom-developed program (*Labview 8.0, National Instrument*). This program also triggered the MRA sequence to synchronise automatically the angiographic acquisitions with dynamic cuffing.

NMR determination of local vascular resistances in murine skeletal muscle: Perfusion and angiography sequences were run alternatively in the leg and in the tail respectively. Corresponding signals were collected separately at rest. Depending on which NMR sequence was running, the ^{1}H receiver chain was automatically switched to the appropriate receiver coil via a fast power switch device dedicated to high radiofrequencies applications (*ES0309-100, Enon)*. Power switch was activated with a TTL signal triggered by the *Bruker* NMR sequences. Vascular resistances of the investigated muscle were obtained by dividing perfusion value by mean systemic arterial pressure value.

Protocol: Ten db/db and control mice were subjected to a protocol consisting of: one minute of dynamic MRA to determine BP values, followed by a 18-second perfusion imaging module repeated over 8min, and terminated with a second NMR module of BP determination.

Results:

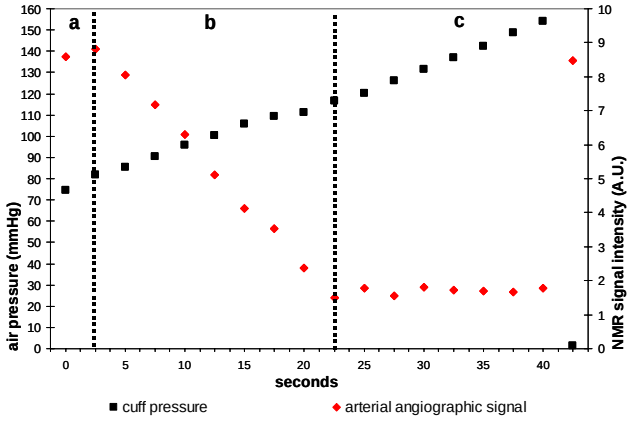

Fig.1: typical time-courses of tail cuff inflation (left scale) and corresponding angiographic arterial signal (right scale):
a-When cuff pressure was lower than arterial BP, the corresponding angiographic signal was roughly constant over time.
b-When cuff pressure was between diastolic and systolic BP, the angiographic signal progressively decreased. Diastolic and systolic BP values were given by the inflexion points of the arterial time-course profile (diastolic BP= 81mmHg, systolic BP: 117mmHg).
c-When tail cuff pressure was greater than systolic BP, caudal arteries were occluded and angiographic signal was minimal.

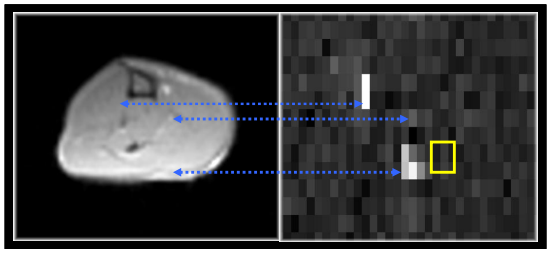

Fig.2: Anatomical transverse section of a control mouse left leg (left) (multislice spin echo: TR=705ms, TE=11ms, resolution: 195x195mm²) and the corresponding perfusion-weighted image (right) with one typical ROI (yellow line) drawn in gastrocnemius muscle where perfusion was measured. Dotted blue lines highlight vessels easily noticeable in perfusion image as hyper intensities (bottom-up: small saphenous vein, anterior and posterior vascularisations).

	systolic BP	diastolic BP	mean BP	tissue perfusion	vascular resistances
	mmHg			ml.min^{-1}.100g^{-1}	mmHg.min.100g.ml^{-1}
db/db (n=10)	79±9*	63±8*	69±8*	6±3*	13,4±6,2*
lean (n=10)	102±14	83±14	89±14	15±4	6,6±1,8

Table1: arterial BP pressure and calf muscle perfusion determined by NMR and calculated vascular resistances at rest in db/db mice vs control (n=10). (*P<0.04, Student test)

Conclusion:
We proposed a non-invasive functional investigation of muscular microcirculation in mice using a new dynamic and multiparametric 1H-NMR approach. We measured automatically local tissue perfusion, systemic blood pressure and determined the corresponding vascular resistances in mice during the same NMR examination. As an application, we showed *in vivo,* that peripheral vascular resistances were increased in the skeletal muscle of db/db mice suffering from type-2 diabetes mellitus, as expected according to previous study[3]. This new NMR method can be used to explore microcirculation control in various mouse models.

Reference: 1-Ménard J. et al. ISMRM 2007 abs. 1492_2-Bertoldi, D. et al., *JMRI* 2003 **18**(4): author reply 515-6_3-Bagi, Z and al., Arterioscler. Thromb. Vasc. Biol. 2005 1610-6.