A newly strictly non-invasive experimental device allowing repeated MR investigations of exercising hindlimb mouse muscles at ultra-high field (11.75T)

J. GONDIN[1], C. VILMEN[1], P. J. COZZONE[1], G. DUHAMEL[1], and D. BENDAHAN[1]

[1]Centre de Résonance Magnétique Biologique et Médicale (CRMBM) - UMR CNRS 6612, Faculté de Médecine - Université de la Méditerranée, Marseille, France

Introduction

Transgenic mouse models of skeletal muscle diseases are attractive tools which should provide new insights into a better understanding of muscle physiology and pathophysiology. Magnetic resonance imaging (MRI) is currently considered as a powerful and suitable non-invasive investigative technique of skeletal muscle structure and function *in vivo*. While conventional T_1 and T_2-weighted MR images can easily provide important clues regarding resting muscle (e.g., muscle volume), exercising muscle can be investigated using more sophisticated MR techniques such as T_2 mapping, blood oxygenation level-dependent contrast or arterial spin labelling (ASL). Given that all these techniques are limited by their low sensitivity, they would benefit from the larger signal-to-noise ratio (SNR) at ultra-high field (>7T)[1] as long as muscle exercise can be made possible in ultra-high field magnet. This is actually challenging given the size of the magnet bores (typically ~90 mm) and the necessity to electrically stimulate muscle and to simultaneously record force production. On the basis of our long-standing experience in the design of ergometers for small animals non invasive investigations[2,3], we have constructed a strictly non-invasive experimental setup adapted to an ultra-high field MR system and report in the present work the results of MRI investigation and force production measurements in exercising hindlimb mouse muscles at ultra-high field.

Methods

Description of the setup: MR experiments were performed at 11.75T (Bruker Avance System, 89 mm wide bore). The experimental setup (*Fig. 1*) was composed of a home-built cylindrical Perspex cradle (29.8 mm diameter) which fit into a 30 mm-diameter transmitting/receiving birdcage coil (Micro2.5 probe; Bruker). It incorporates four distinct components allowing non-invasive muscle stimulation, force output measurements, prolonged anaesthesia and respiration monitoring.

Muscle stimulation: Transcutenous electrical stimulation was performed by two rod-shaped 1.5 mm-diameter surface electrodes incorporated into the cradle and connected to an electrical stimulator. One electrode was located at the heel level and was in contact with the Achilles tendon of the mouse. The second electrode was inserted in a designed piecework and was placed at the knee level so that the leg was firmly immobilized.

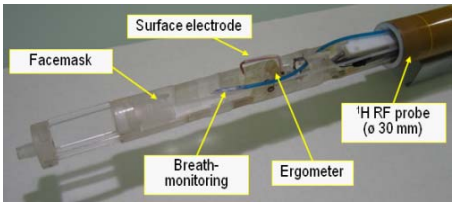


Fig. 1 *– Experimental setup allowing the complete non-invasive investigation of skeletal muscle function in mouse using ultra-high field MRI.*

Force measurements: Isometric force was measured with a home-built ergometer consisting of a 9×15 mm foot pedal coupled to a force transducer. Given the ultra-high field, the transducer was necessarily located far from the magnetic center and the force applied on the pedal was transmitted to the strain gauge through a 650 mm length inextensible nylon thread positioned perpendicularly to the axis of both the pedal and the transducer. The resulting output signal was amplified with a home-built amplifier and converted to a digital signal which was recorded on a PC using the WinATS software (Sysma, France).

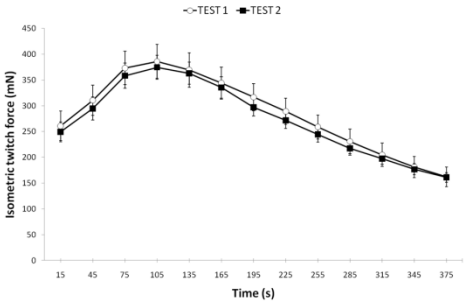
Monitoring techniques: A home-built facemask was incorporated into the cradle and was used to maintain prolonged anaesthesia throughout the experiment while respiration was monitored using a pressure sensor positioned under the mouse abdomen (SA instruments Inc., NY, USA).

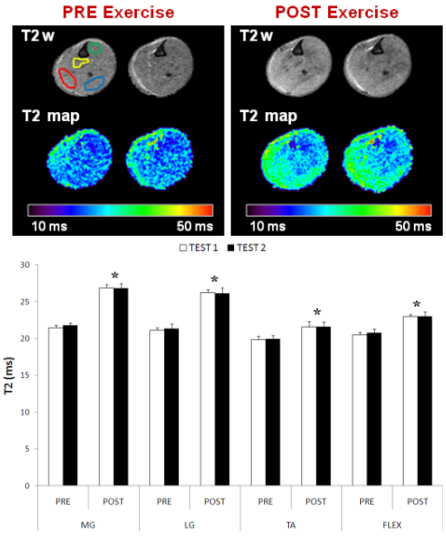
Experimental protocol: Experiments were performed on 10-week old C57BL/6 healthy mice (n= 6) weighing ~25 g. The stimulation protocol consisted of single twitches delivered under isometric conditions at ~1.85 Hz during 6.5 min. Mice were tested twice over a one-week period to investigate the reliability of measurements for mechanical performance and T_2 changes associated to the stimulation protocol. ADC maps were acquired during the exercise session. Test-retest reliability was analyzed using coefficient of variation (CV) for both force measurements and T_2 values

MRI: The following acquisition parameters were used: T_2: MSME sequence; TE= from 7.5 to 45.5 ms; TR=800ms; Matrix=128x128; FOV=2x2cm²; 10 slices. *ADC*: 2-shot SE-EPI ; TE=24.8ms ; Matrix 128x128 ; FOV=2x2cm² ; diffusion gradient in Z-direction ; 8 b-values: 0, 10, 30, 50, 100, 200, 400, 700 s/mm² ; 3 slices (1mm) ; 10 NEX. MR acquisitions were synchronized to the stimulation protocol. Mean T_2 values for the medial gastrocnemius (MG), lateral gastrocnemius (LG), tibialis anterior (TA) and flexor (FLEX) muscles were measured on T_2 maps by manually outlining regions of interest using a custom-written image analysis program.

Results

- High reproducibility of both ***mechanical performance*** (CV = 9.4%; *Fig. 2, Left*) and ***T_2 values*** (CV = 1.0% in *MG*; CV = 1.5% in *LG*; CV = 1.1% in *TA*; CV = 1.3% in *FLEX*; *Fig. 2, Middle, Bottom*).
- Preferential activation of the gastrocnemius muscles (i.e., MG and LG) during the exercise (*Fig. 2, Middle*)
- Good image quality, free of motion artifact for the acquisition performed during the exercise (*Fig. 2, Right*)





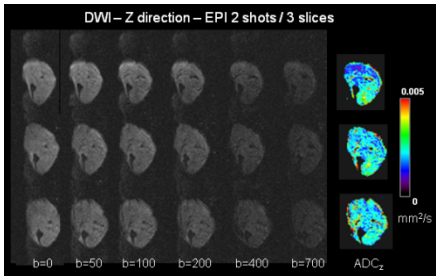


Fig. 2 *– Isometric force recorded in hindlimb mouse muscles during the exercise on two testing sessions (Left). Representative T_2w-images and the resulting T_2 maps (Middle, Top) and T_2 values (Middle, Bottom) recorded before (PRE) and after (POST) the exercise on two testing sessions. ROIs drawn in red, blue, green and yellow correspond to the MG, LG, TA and FLEX muscles, respectively. DW SE-EPI images (Right) and resulting ADC maps acquired during the stimulation protocol. * Significantly different from PRE*

Discussion

This newly strictly non-invasive device allowed highly reproducible measurements of both force production and muscle activation (T_2 changes) in exercising hindlimb mouse muscles within an ultra-high field magnet. Neither side effect of the stimulation device positioned inside the RF probe nor alteration of the SNR was observed on the different types of MR images. Although a highly motion-sensitive imaging sequence was used (2-shot EPI), ADC experiments performed during the exercise session and synchronized to the stimulation pattern were of high quality and free of motion artifacts, opening then the perspective to perform more complete protocols during exercise. This would include muscle perfusion imaging (with ASL) combined with ^{31}P-magnetic resonance spectroscopy. On that basis, our setup appears as a suitable tool for future ultra-high field functional MR investigations of exercising hindlimb mouse muscles.

References : 1. Chang *et al. Semin Musculoskelet Radiol* (14):269-278; **2010**. 2. Giannesini *et al. Magn Reson Med* (54):1058-1064; **2005**. 3. Giannesini *et al. Magn Reson Med* (64):262-270; **2010**.