An MR compatible setup for simultaneous measurements of skeletal muscle performance and ^{31}P MRS in the mouse

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Introduction. Studies of *in vivo* muscle function often combine dynamic ^{31}P MRS and measurements of muscle performance. In mice, however, these studies are challenging due to the small size of the animal. Common procedures for measuring skeletal muscle force in mice involve removal of the Achilles tendon [1]. Disadvantages of this method are interference with muscle physiology and its invasive nature.
In this study we present an MR compatible setup, which has been designed to measure both isometric skeletal muscle performance *in vivo* and ^{31}P MRS simultaneously during and after contraction of skeletal muscle, avoiding detachment of the Achilles tendon. A dual coil setup has been to measure both ^{31}P and ^{1}H without disturbing the muscle force measurements. This setup enables reproducible positioning of the hind leg of the mouse with respect to both coils and the force transducer.

Materials and methods. Contraction of the calf muscle was achieved by stimulation of the sciatic nerve in the upper leg. After anesthesia, a minor incision was made in the upper leg of the mouse. To avoid contraction of the dorsal flexors, the tibia nerve was cut. Then an electrode was tunneled through the upper leg and connected to the sciatic nerve by stitching the electrode to the surrounding tissue leaving the sciatic nerve in its natural position. A second electrode was fed through the skin near the Achilles tendon, functioning as ground reference for the stimulation pulses.
Increasing the current through the sciatic nerve until no further increase in force was achieved, was used to set the optimal stimulation current. Stimulation of the skeletal muscle was achieved by a pulse train of 250 ms at 150 Hz.

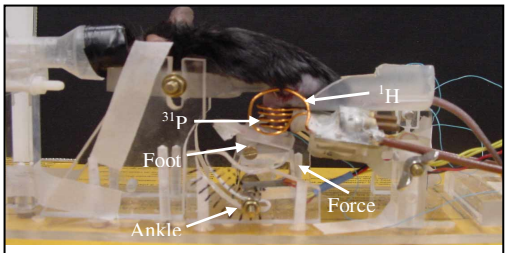

Figure 2: complete setup with the coils, force transducer, foot fixation and ankle rotation.

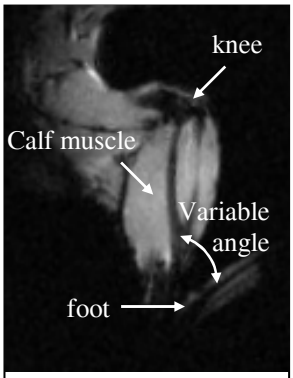

Figure 1: MR Image of the hind leg in the setup showing knee angle, calf muscle and rotating foot

Muscle performance was measured by a custom-made MR compatible force transducer. The force transducer was designed to fit into the mechanical setup, leaving enough space for the MR coil setup. Force was measured by attaching 4 strain gauges onto the force transducer in a Wheatstone bridge design, achieving optimal force transduction. For noise reduction both stimulation and force measurement lines were filtered with low pass filters giving a damping of 60 dB at 120 MHz.
The hind leg of the mouse was fixed onto the force transducer by fixing the foot in a close fitting cradle and fixing the knee into a cavity in a Perspex plate. Fixing of the hind leg was done so that the knee was in a 90 degree angle, providing optimal force transduction. The angle of the ankle could be changed by rotating the force transducer to set the optimal length of the calf muscle (figure 1).
A dual coil setup was built consisting of a ^{31}P coil (121.5 MHz) for unlocalized ^{31}P MRS and a ^{1}H coil (300.2 MHz) for reference imaging and localized shimming of the hind leg. To avoid coupling of the coils, the field orientation of the coils was constructed perpendicularly. For SNR optimization a 4 turn funnel shaped solenoid coil was designed as ^{31}P coil so that the highest Q-factor (Qloaded = 120) and B1 field homogeneity was achieved. The funnel shape was made so that the coil would give a tight fit around the hind leg without interfering the muscle movement during contraction. The ^{1}H coil was designed as a surface coil to not interfere with the ^{31}P coil or the force measurement.
The complete setup (figure 2) was designed to enable a prone position for the mouse with the lower leg vertical into the NMR scanner for the best possible natural position while keeping both coils perpendicular to the main magnetic field. Body temperature was monitored by an optical thermometer and kept constant by a warm water bed. Anesthesia was performed with isoflurane (2%) through a nose cap and breathing was monitored by a movement sensor. A user interface was designed for controlling the stimulation, measuring the force transduction and synchronizing the measurements to the spectrometer and pulse sequences using LabView. The setup was tested on 8 wild type mice in a 7T horizontal scanner. ^{31}P MR spectra (Tr = 4900 ms, 192 Avg.) were acquired and data was analyzed using jMRUI.

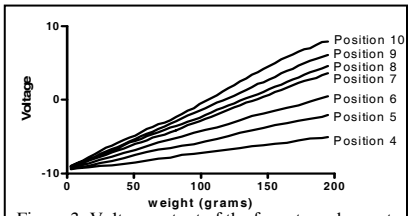

Figure 3: Voltage output of the force transducer at different forces, measured with reference weights. Each position represents a different ankle angle.

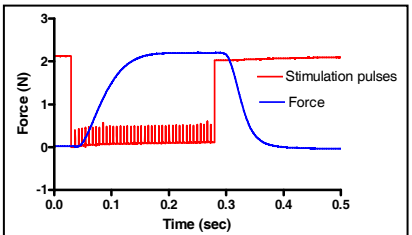

Figure 4: Typical force signal after stimulation with a pulse train of 250 ms at 150 Hz.

Results. A calibration curve of the force transducer was measured for calculating the force of the skeletal muscle at different ankle angles (figure 3). Angles were chosen within a normal physical range varying from 60 to 120 degrees. A force signal was recorded for measuring the force of the calf muscle (figure 4) resulting in a mean force of 1.98 ± 0.49 N. Automatic disconnection of the electrodes causes the apparent high current (see figure 4) when no stimulation is given. This disconnection excluded the possibility of leaky currents when the skeletal muscle was in rest.
Measurements with the 31P coil during a stimulation protocol resulted in representative 31P spectra both before and directly after contraction (figure 5). This resulted in a PCr/ATP ratio (uncorrected for T1) of 2.6 ± 0.3 before and 2.0 ± 0.4 directly after contraction.

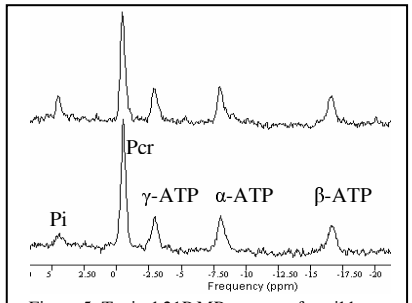

Figure 5: Typical 31P MR spectra of a wild type mouse before (bottom) and shortly after contraction (top). Note the decrease in PCr and concomitant increase in Pi after contraction.

Conclusion and discussion. A setup for measuring both maximal isometric skeletal muscle performance *in vivo* and ^{31}P MRS simultaneously was designed and evaluated. With this design a protocol for measuring force and PCr/ATP ratios with ^{31}P MRS can be measured with high reproducibility in an *in situ* situation with a minimal invasive technique.
Another setup that avoids detachment of the Achilles tendon and combines MR measurements with the assessment of muscle performance has been developed for 31P MRS in rats in a strictly non-invasive way [2]. A device, which enables repeated measurements in mice using an implantable electrode has been realized only for MR imaging [3]. The current setup enables, for the first time in mice, dynamic ^{31}P MRS combined with measurements of skeletal muscle performance without removal of the Achilles tendon.
In the future applying an implantable electrode [4] or stimulating strictly non-invasively [2] would enable longitudinal follow up studies.

References. [1] Giannesini et al. (MAGMA 17: 2004), [2] Giannesini et al. (MRM 54: 2005), [3] Drost et al. (Eur J Physiol 447: 2003), [4] Warren et al. (J Appl Physiol 84: 1998)