Gated ^{31}P MRS acquisition during steady state electrical stimulation of mouse skeletal muscle enables determination of contractile ATP cost and phosphocreatine recovery time

H. E. Kan[1], A. Veltien[1], H. Arnts[2], B. Luijten[1], A. de Haan[3], B. Wieringa[4], and A. Heerschap[1]

[1]Radiology, Radboud University Nijmegen Medical Center, Nijmegen, Netherlands, [2]Central Animal Laboratory, Radboud University Nijmegen Medical Center, Nijmegen, Netherlands, [3]Department of Human Movement Sciences, Free university, Amsterdam, Netherlands, [4]Cell Biology, Radboud University Nijmegen Medical Center, Nijmegen, Netherlands

Introduction. In humans, dynamic ^{31}P MRS combined with measurements of skeletal muscle performance have been used extensively to study various aspects of skeletal muscle function *in vivo*, like aerobic capacity, intramuscular pH regulation and ATP contractile cost [1]. In mice however, these studies are challenging due to the small size of the animal, resulting in a low signal to noise ratio (SNR) and technical challenges in force measurements and electrical stimulation of the nerve. In the present study, we developed a measurement protocol with gated ^{31}P MRS acquisition to enable determination of ATP contractile cost and PCr recovery time at the same time in mice, based on a protocol recently developed for humans [2]. Skeletal muscle force of the plantar flexor muscle complex was assessed *in situ*, thereby avoiding common more invasive force measurements using detachment of the Achilles tendon [3].

Methods. Unlocalized ^{31}P MRS (7T, horizontal bore magnet) and measurements of skeletal muscle performance were performed in 8 control mice (Con) and 3 mice deficient in cytosolic creatine kinase (M-CK) and adenylate kinase (MAK$^{-/-}$). Mice were anesthetized with isoflurane (2%) and prepared for *in situ* stimulation of the left plantar flexors by exposing the sciatic nerve through a small incision at the hip. After severing the tibial nerve branch to avoid contraction of the dorsal flexors, a wire electrode (tensile flex UT3607TF) was placed near the sciatic nerve, attached to the surrounding tissue and tunneled through the upper leg to avoid displacement. A second electrode was fed through the skin near the Achilles tendon. Thereafter, the mouse was placed in the experimental setup with its ankle rigidly fixed on a rotatable force transducer, its knee rigidly fixed at ~90 degrees by a perspex plate and its hind leg placed in a solenoid coil for ^{31}P MR measurements. A ^{1}H surface coil was used for imaging and shimming and body temperature was maintained by a warm water blanket.

Before the MR experiment optimal ankle angle, i.e. the ankle angle which yielded the highest force, was determined using tetanic isometric contractions at different ankle angles (250 ms, 150 Hz, optimal current). Thereafter, the setup was placed inside the magnet and three ^{31}P MR spectra (TR=4900 ms, 64 averages) were acquired during resting conditions. Subsequently, the stimulation protocol was started consisting of one tetanic contraction (250 ms, 150 Hz) every 30 s for 35 minutes (total of 70 contractions). Six ^{31}P MR acquisitions were performed during each contraction cycle of 30 seconds, the first one 250 ms after the last stimulation pulse.

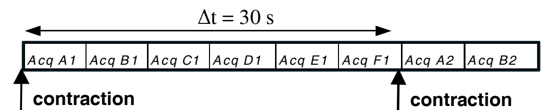

Figure 1. Schematic of the combined gated (averaging Acq A1, A2, etc.) and sequential (averaging Acq A1,B1, etc.) data acquisition. Maximal isometric contractions were performed with a time interval Δt of 30 s.

Data analysis of the ^{31}P MR signals was performed by retrospectively averaging single MR acquisitions sequentially (64 subsequent scans, i.e. A1, B1, etc) or gated (65 corresponding scans, i.e. A1, A2, etc)(fig1). The resulting MR spectra (6 for both sequentially and gated averaged MR spectra) were analyzed using jMRUI. The time constant for phosphocreatine (PCr) recovery (τ) was computed as follows: $\tau = -\Delta t/\ln(D/[D+Q])$ where Q is the decrease in PCr after contraction or ATP contractile cost, D is the additional steady-state drop in PCr below resting values and Δt is the interval between the contractions [2].Torque signals were digitized, filtered and analyzed for maximal force.

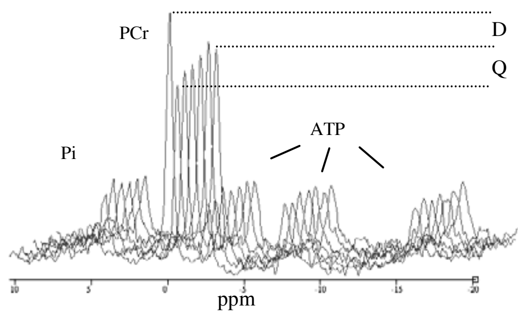

Figure 2. Gated ^{31}P MR spectra of a Con mouse before (front spectrum) and during the gated protocol. The decrease in PCr from steady state is represented by D, the additional decrease due to contraction by Q.

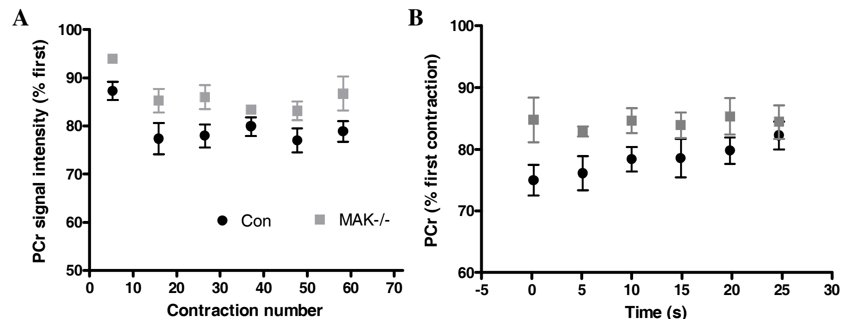

Figure 3. Time course of changes in PCr signal intensity after sequential (A) and gated (B) averaging of the ^{31}P MR spectra. Con mice are shown by black diamonds, MAK$^{-/-}$ mice by grey squares.

Results. Stimulation of the sciatic nerve every 30 s resulted in a decline and subsequent recovery of PCr in Con mice (Fig 2). Sequential averaging of ^{31}P MR spectra showed a steady state decrease in PCr levels after 10 contractions in Con animals to 78.9±6% of resting values during the last contractions (Fig 3A). Muscle force showed a similar tendency and decreased to 77±13 % of the first contraction at the end of the protocol. Gated averaging of the MR spectra showed a steady state decrease in PCr from resting conditions (D in fig 2) of 17.8±6 % in Con mice and 15.6±4.7 % in MAK$^{-/-}$ (Fig 3B). The additional drop in PCr due to contraction was 7.5 ± 2 % in Con and negligible in MAK$^{-/-}$ mice (Fig 3B). These values yielded a τ for Con animals of 89±34 s. Assuming an PCr concentration of 30 mM, the ATP contractile cost in Con animals was 2.3 mM/per contraction.

Conclusion and discussion. In this study we present for the first time in mice a combined gated and sequential protocol for data analysis based on a recently described method for estimation of ATP contractile cost and PCr recovery time in humans [2]. This stimulation protocol enables steady state levels of PCr without dramatic loss of force during 70 maximal isometric contractions. The gated data acquisition enabled the determination of rapid PCr changes that would otherwise go unnoticed due to intrinsic low SNR in mouse skeletal muscle. This analysis showed a considerably smaller decrease in PCr levels in MAK$^{-/-}$ mice, in agreement with results on M-CK deficient mice [4], a direct reflection of the eminent role of CK in "burst activity".

References. [1]Chance, B. et al. *NMR Biomed*, 2006. [2] Slade, J.M. et al. *NMR Biomed*, 2006. [3] Giannesini, B. et al. MAGMA, 2004. [4] Roman, B.B. et al. *Am J Physiol Cell Physiol,* 2002