An NMR-compatible, minimally invasive electrical stimulation method to study rat skeletal muscle function *in vivo*

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Introduction

Both MR imaging (MRI) and MR spectroscopy (MRS) have proven to be valuable tools for the non-invasive study of skeletal muscle, in humans as well as in animals (1). ^{31}P MRS is widely used to monitor the dynamics of energy metabolism during and after muscle contractions. When ^{31}P MRS is applied to skeletal muscle in anaesthetized animals, the muscle contractions have to be induced by electrical stimulation. Consequently, the invasive character of different stimulation methods often undermines the non-invasive nature of MRI and MRS, hampering longitudinal studies.

We present an NMR-compatible, minimally invasive subcutaneous electrical stimulation procedure for rat lower hind limb that induces isolated contractions of the dorsal flexor muscles. The set-up's specificity for the dorsal flexor muscles (extensor digitorum longus (EDL) and tibialis anterior muscle (TA)) is shown by the contraction-induced T_2 increase in ^{1}H MRI and its applicability for longitudinal studies is demonstrated by a longitudinal ^{31}P MRS study in diabetic ZDF and control rats.

Materials and methods

Dynamic ^{31}P MRS before, during and after muscle contractions was applied in ZDF fa/fa rats (n = 10) and control fa/+ (n = 11) animals at different ages (6, 12 and 18 weeks of age) representative for different stages in the pathogenesis of type 2 diabetes. Contraction-induced T_2 increase was measured in one Wistar rat (~ one year of age). All NMR experiments were performed on a 6.3 Tesla horizontal Bruker MR system. A set-up with a combination of a circular ^{1}H surface coil and an ellipsoid ^{31}P surface coil was used. Shimming was performed on the water signal and subsequently the acquisition of T_2 weighted images or ^{31}P MR spectra was started.

The in-house built stimulation electrodes were made of 2 platinum strips (1.2 × 8 mm^2) that were soldered to flexible multi-stranded copper wire (Cooner Wire, AS 999-30, Chatsworth, Ca, USA) (figure 1a). Before the subcutaneous insertion of the electrodes, the rats were anaesthetized and positioned supine in a cradle. This cradle had a support for one lower hind limb and a footplate. The hind limb support and footplate were constructed in such a way that the angle of both the hip and the knee was ~ 90°. Two small incisions were made (~ 1.5 mm wide) along the nerve trajectory of the *N. peroneus communis*. Two skin pockets were made by blunt preparation, both electrodes were inserted and the wounds were closed with a single stitch (6.0, Prolene, Ethicon, Inc. NJ, USA) (figure 1b).

T_2 weighted images were made using a multi-echo spin-echo sequence with 6 different echo times (TR/TE = 2 s/11, 21, 32, 43, 53 and 64 ms, averages = 2, matrix = 256 × 192, FOV = 30 × 30 mm^2, in plane resolution = 117 × 156 µm^2, slice thickness = 2 mm). Imaging was performed before electrical stimulation and 10 minutes after the onset of electrical stimulation. To prevent a decrease in T_2 prolongation during the acquisition of the image, the stimulation protocol was continued during the delays in the imaging sequence. The stimulation protocol consisted of block pulses with a length of 10 ms, applied every 400 ms and carried out till the end of the image acquisition. Two T_2 weighted images (TE = 32 ms) were subtracted to highlight the region that had the most signal increase due to prolongation of T_2. ROI's were drawn by hand on a pre-stimulation T_2 weighted image (TE = 11 ms) representing different muscles of the lower hind limb and T_2 was calculated based on the signal acquired with the 6 different TE's.

^{31}P spectra were acquired applying an adiabatic pulse with a flip angle of 90 degrees. A time series of spectra (TR = 5 s, 4 averages) was acquired before, during and after the muscle contractions. The muscle exercise consisted of a series of stimulation pulses, applied every second, for a duration of 2 min. The stimulation pulse length was 100 ms, the stimulation frequency was 80 Hz and the voltage varied between 2 and 4 V. ^{31}P spectra were fitted in the time domain by using a nonlinear least squares algorithm (AMARES) in the jMRUI software package (2).

Results & discussion

The subcutaneous electrical stimulation procedure was well tolerated by the animals, as they restored their normal body weight and food intake within 2 days after an MRS session including the electrical stimulation procedure. The small incisions through the skin healed fast, allowing repetitive measurements within ~ 10 days.

The analysis of the T_2 weighted images showed an area of increased signal intensity after electrical stimulation (figure 1c-e) that corresponded with the anatomical location of the TA and EDL muscle. The calculation of the T_2 values in the different regions of the hind limb confirmed an increased T_2 enhancement in the region of both TA and EDL, whereas no change in T_2 was observed in other muscles of the hind limb, thus confirming the specificity of the stimulation procedure for the dorsal flexor muscles (figure 1f and table 1). Figure 2 shows a typical example of a time series of ^{31}P MRS spectra measured in the dorsal flexor muscle complex. The line width of the PCr peak varied between 12 and 18 Hz, comparable to measurements without electrodes indicating that the electrodes did not induce local disturbances of the magnetic field.^{31}P MRS data from the study in ZDF rats are presented in table 2 showing that the end-exercise status of PCr and pH measured at different time points can be reproduced, confirming the applicability of the present electrical stimulation method for longitudinal study designs.

Conclusion

We present an NMR-compatible, minimally invasive electrical stimulation procedure for the dorsal flexor muscles of the rat that 1) was well tolerated by the animals 2) induced highly specific muscle contractions and 3) proved to be applicable in longitudinal study designs. Further research is recommended to investigate the feasibility of this method to stimulate other muscles of the rat and its possible use in mouse.

References 1) Prompers JJ, *et al*., NMR in Biomedicine, 2006. 2) Vanhamme L, *et al.,* J Magn Reson, 1997.

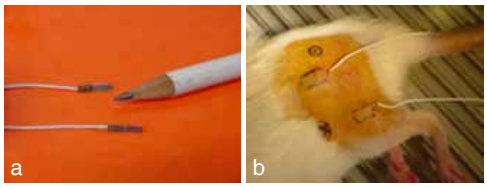

Table 1	T_2 Pre ± SD	T_2 Post ± SD
TA	20.7 ± 2.0	24.4 ± 3.2
EDL	19.5 ± 2.9	22.6 ± 4.4
SOL	21.1 ± 1.5	20.9 ± 1.3
TP & FDl	22.5 ± 3.3	22.9 ± 3.3
GL	22.5 ± 2.9	22.4 ± 3.0

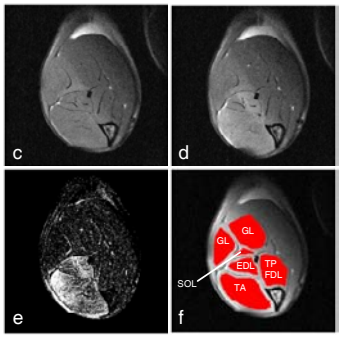

Figure 1: a) platinum electrodes, b) subcutaneous implantation of the electrodes in the anaesthetized rat, c) pre-stimulation T_2 weighted image, TE = 32.9 ms. d) post-stimulation T_2 weighted image, TE = 32.9 ms, e) subtraction image: d – c, f) ROI's depicted on pre-stimulation T_2 weighted image, TE = 10.6 ms, TA = m. tibialis anterior, EDL = m. extensor digitorum longus, TP = m. tibialis posterior, FDL = m. flexor digitorum longus, SOL = m. soleus, GL = m. gastrocnemius lateralis. **Table 1:** averaged T_2 values calculated from the corresponding ROI's depicted in fig. 1f. T_2 pre and post = average T_2 value before and after stimulation, respectively.

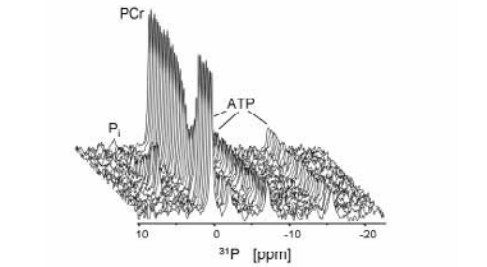

Table 2	6 weeks		12 weeks		18 weeks	
	Lean	Fatty	Lean	Fatty	Lean	Fatty
PCr rest	32.5 ± 1.5	31.7 ± 1.2	38.5 ± .09	40.2 ± 1.2	39.4 ± 1.6	39.1 ± 2.0
PCr end	17.0 ± 2.3	16.7 ± 2.3	19.9 ± 2.0	21.4 ± 2.3	21.8 ± 1.6	19.5 ± 1.7
pH end	6.91 ± 0.06	6.86 ± 0.10	7.02 ± 0.10	6.98 ± 0.08	6.96 ± 0.06	6.93 ± 0.06

Figure 2: example of a time series of spectra measured in a rat TA, time resolution = 20 s, stimulation duration = 2 min. **Table 2:** selection of data from a longitudinal study in ZDF rats. PCr rest = phosphocreatine concentration at rest (mM), PCr end = phosphocreatine concentration at the end of the 2 min stimulation protocol (mM). pH end = pH at the end of the 2 min stimulation protocol. Lean = fa/+ ZDF rat, Fatty = fa/fa ZDF rat.