Perfusion, BOLD and bioenergetics changes after plasmid electrotransfer in mouse leg skeletal muscle assessed by multiparametric functional (mpf-) NMR *in vivo*

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INTRODUCTION: *In vivo* gene electrotransfer (ET) is frequently used in preclinical gene therapy studies. The transient permeation of cell membranes facilitates therapeutic material transfection without resorting to viral vectors. Over the past decade, many studies have attempted to optimize ET protocols to increase efficiency while reducing variability of transgene expression and muscle damage. Most of them reported histological evidences of induced muscle degeneration and completion of subsequent regeneration within 15 days [1,2]. The functional consequences of this process have rarely been addressed [3], which may in part reflect the lack of appropriate methods of investigation. Yet, for an accurate assessment of gene therapy protocols, it is of paramount importance to properly characterize the functional alterations induced by the procedure itself, as it may interfere with the therapy. NMR imaging and spectroscopy allow the assessment of a number of key functional parameters *in vivo*. However, mouse investigation is still challenging and measures are usually collected separately over repeated experiments. Recently, we developed a multiparametric functional (mpf-)NMR protocol including strength measurement [4] which simultaneously acquires arterial spin labeling (ASL) imaging of tissue perfusion and blood oxygenation level dependent (BOLD) contrast, as well as dynamic ^{31}P spectroscopy of phosphocreatine (PCr) kinetics and pH measurement during electrically stimulated (ES) exercise and recovery in mice leg. In this work, mpf-NMR was used to quantitatively and non-invasively investigate long-term changes in mice leg muscle function after ET of an empty plasmid.

METHODS: **Plasmid electro-transfer:** Both legs of 9 mice (FVB, 8-week-old) were injected in 5 different sites with an empty plasmid solution using a 30-gauge needle (30µl; 1 µg/µl; pM1 Expression Vector (Roche Molecular Biochemicals, Indianapolis, IN) (pM1). ET was performed using plate electrodes (8 pulses 20 ms, 200V/cm, 2 Hz, [5]). Ten FVB mice were taken as controls (Ctr). ***In vivo* mpf-NMR evaluation:** Animals were subjected to mpf-NMR 15 days after ET, in a 4T Bruker Biospec NMR spectrometer equipped with a 20 cm diameter 200 mT/m gradient insert. *Exercise:* The ES protocol consisted of intermittent isometric 0.5s tetanic contraction every 2.5 s for 2 min (pulse: 120µs, 50Hz, 2mA, COMPEX stimulator, subcutaneous electrodes). *Strength measurements:* A custom-built NMR-compatible ergometer was used. Signal was adequately filtered and amplified, then processed in Labview. *Perfusion/BOLD:* Pulsed ASL-RARE images were obtained every 10 s using the SATIR variant [6] (inter-echoes time= 2.9 s, RARE factor= 32, FOV=5x2 cm, matrix= 128x32). Consecutive pairs of SATIR images were first subtracted for perfusion calculation, and then averaged to extract BOLD contrast as described in a previous study [7]. *Gated ^{31}P-NMRS:* Phosphorus metabolites of the leg were probed using a saddle shaped coil (diameter: 1 cm/ length: 1cm). Spectra were acquired at Ernst angle with a repetition time (TR) of 2.5 s and a pulse length of 100 µs. Exercise (2min)-recovery (10 min) episodes were repeated 12 times to allow the gated summation of results at steady state [5] and increase ^{31}P signal-to-noise. PCr recovery was fitted to a mono-exponential function and the time constant τPCr used as an index of oxidative capacities. *Interleaved acquisitions*: ASL imaging and ^{31}P spectroscopy were interleaved using the Bruker MultiScanControl tool, so that 1 perfusion/BOLD image and 4 ^{31}P spectra were obtained every 10 s. Acquisitions were triggered to ES, to allow perfusion measurement even during exercise. **Histological analysis:** Gastrocnemius muscles were taken and stained for succinate dehydrogenase (SDH) activity. Immunostaining of anti-CD34 antibodies against endothelial cells was used to estimate capillary contact per fiber.

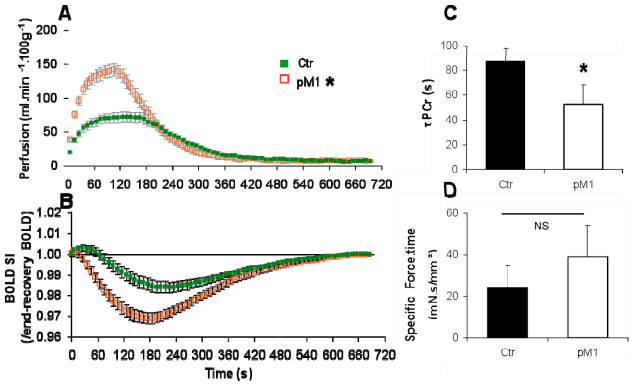

*Figure 1. Perfusion (A), BOLD (B), τPCr (C), and force (D) acquired simultaneously in control and pM1 electrotransfered mice leg. *p<0.05.*

RESULTS: Fifteen days after ET, the cross sectional area assessed from anatomical MR images was decreased by 11% in pM1 group (Ctr: 50±3, pM1: 44±2 mm²; p=0.0007). Although PCr consumption was greater in pM1 (Ctr: 58±12, pM1: 66±11%; p=0.002), neither specific force-time integral (Figure 1D) nor end-exercise pH (Ctr: 7.03±0.03, pM1: 7.01±0.02) differed, while τPCr was significantly shortened in pM1 (Ctr: 87±10, pM1: 53±15 s; p=0.05; Figure 1C) and [PCr]/[ATP-β] identical at rest, indicating increased oxidative capacities. In parallel, maximal perfusion (Ctr: 83±22, pM1: 145±27 ml.min^{-1}.100g^{-1}; p<0.0001) and perfusion integral at exercise (p<0.0001; Figure 1A) were almost doubled in pM1, and capillary oxygenation significantly decreased as shown by a more pronounced negative BOLD (Figure 1B). Histology revealed numerous fibers with central nuclei in pM1, attesting of the regeneration process, but no difference in fiber type distribution (relative SDH positive area- Ctr: 33.9±5.2, pM1: 30.1±3.3%; p=0.2) or in capillary contact per fiber type was found.

CONCLUSION: In this standard mouse model of plasmid ET, a ~10% loss in muscle mass was accompanied by important alterations of both vascular and bioenergetics dynamics at exercise, which may prove beneficial to muscle function. Fifteen days post-ET, a time conventionally considered sufficient for muscle to recover [1,2,3], these changes could not be correlated to any shift in fiber type nor to a remodeling of vasculature at the capillary level, though paralleled a degeneration/regeneration process. Our mpf-NMR protocol provided a new insight into the functional consequences of ET as is frequently performed in mouse muscle and constitutes a powerful tool for the optimization of ET protocols and more globally the longitudinal assessment of preclinical gene therapy.

References: [1]Mathiesen, Gene Therapy 1999 [2]Hartikka, et al. Mol Therapy 2001 [3]Schertzer et al. Molecular Therapy 2005 [4] Baligand, et al. ESMRMB 2008 [5]Bloquel, et al. J Gene Med 2004 [6]Raynaud, et al. Mag Reson Med 2001 [7]Duteil, et al. Mag Reson Med 2005.