In vivo diffusion MR study at 7T of hindlimb muscles in a mouse model of Duchenne muscular dystrophy

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TARGET AUDIENCE

MRI physicist, radiologist and biologist interested in skeletal muscle diseases.

INTRODUCTION

In this study we aimed to investigate and evaluate the differences in the microstructure between hindlimb muscles of dystrophic (*mdx*) and wild type (*wt*) mice using diffusion MRI. Diffusion weighted imaging (DWI) protocols were optimized by exploring long diffusion times[1,2] (Δ) suitable for probing the architecture of muscles at the microscopic level with the goal of discriminating diseased from healthy tissue. In addition, histological assessment of the excised hindlimbs was performed in order to determine muscle fiber size of the investigated muscles.

MATERIALS AND METHODS

Male *mdx* (n=17) and wild type (n=17) control mice were used for *in vivo* and *in vitro* investigation of gastrocnemius (GA) muscle microstructure. Mice were grouped according to their age and underwent *in vivo* MRI at 7.5 weeks (Group A, n=6), 22 weeks (Group B, n=6) and 44 weeks (Group C, n=5) of age. MRI measurements were performed using a 7T/21cm scanner (Varian/Magnex Scientific) equipped with a volume coil used as a radio-frequency transceiver. MRI protocol included T2-weighted turbo-spin-echo sequence, multi-slice-multi-echo images for T_2 determination, and DWI measurements performed by using the pulse gradient stimulated echo (PGSTE) sequence (TR=4000ms, TE=20ms, δ=3ms, in-plane-resolution=156x156μm^2). Six Δ values (25, 60, 100, 150, 250, 350 ms) were used at a fixed gradient amplitude (GDiff = 22Gauss/cm) for probing the microstructural differences between the GA muscle in *mdx* and *wt* control mice. For each Δ, DWI measurements were performed by using four b-values ranging from 13 to 2700s/mm^2 with the diffusion gradient applied along the fiber direction (slice-selection direction) as well as across the fiber direction (phase-encoding direction). Apparent diffusion coefficient (ADC) maps were calculated by fitting the signal intensity as a function of b ($S(b)=S(0)\exp(-b\cdot ADC)$). Mean ADC values in the GA muscle of *mdx* and *wt* mice were calculated using ROI-based analysis. Statistical analysis was performed using a two-tailed, unpaired Student's *t*-test. Following MRI, mice were sacrificed and the excised limbs were skinned and fixed in formalin (10%). In addition, *in vitro* measurements were performed on a single fixed hindlimb for each mouse using the same MRI protocol as for the *in vivo* measurements. Finally, fixed limbs were decalcified in EDTA (20%) for 2 weeks at pH 7.4. Paraffin embedded sections (5μm thick) were then cut and stained with H&E. Feret's diameter of each muscle fiber was measured manually using Fiji (ImageJ).

RESULTS

Mean ADC values of GA muscle in *mdx* and *wt* mice were calculated for each Δ with GDiff along the fibers (Table 1, Fig. 1A) as well as GDiff across the fibers (displayed in Fig.1A). Compared to *wt* mice, higher ADC values were found in *mdx* mice along the GA muscle fibers (Table 1). Values were significantly different for long Δ but not for short Δ (Table 1, Group A and Group C). A significant decrease in the diffusivity of water was observed, for each Δ, in both *mdx* and *wt* mice when their age increased from 7.5 to 22 weeks (Table 1, Group A and B). Conversely, no differences were found when mouse age increased from 22 to 44 weeks (Table 1, Group B and C). T_2 relaxation times of GA muscle (not reported) showed a correlation with ADC values in both *mdx* and *wt* mice decreasing with increasing mouse age. In addition, *in vitro* data (not reported) showed the same pattern of diffusivity displayed in Fig 1A with lower ADC values. Preliminary results of muscle fiber size in GA muscle cross-sections have shown higher variability in the distribution of fiber sizes in *mdx* mice compared to *wt* as has been previously reported [3].

Table1. ADC values of gastrocnemius muscle in *mdx* and *wt* mice aged 7.5, 22 and 44 weeks (Group A, B and C, respectively). The reported values were determined with diffusion gradient applied along the fiber direction and by exploring six different Δ. Values are mean ± 1 s.e.m.

Δ (ms)	*ADC* values (10^{-3} mm^2/s)					
	Group A		Group B		Group C	
	mdx	*wt*	*mdx*	*wt*	*mdx*	*wt*
25	1.75 ± 0.04$^{\#}$	1.73 ± 0.04$^{¥\#}$	1.67 ± 0.03$^{¶\#}$	1.46 ± 0.03	1.50 ± 0.02	1.53 ± 0.02
60	1.67 ± 0.05$^{¥\#}$	1.59 ± 0.06$^{¥\#}$	1.52 ± 0.03$^{¶\#}$	1.30 ± 0.03	1.38 ± 0.02	1.36 ± 0.02
100	1.69 ± 0.04$^{¥\#}$	1.59 ± 0.04$^{¥}$	1.53 ± 0.04$^{¶\#}$	1.28 ± 0.03	1.41 ± 0.02¶	1.33 ± 0.02
150			1.48 ± 0.04$^{¶\#}$	1.25 ± 0.03	1.34 ± 0.02¶	1.27 ± 0.02
250	1.57 ± 0.04$^{¶¥\#}$	1.47 ± 0.03$^{¥\#}$	1.46 ± 0.04$^{¶\#}$	1.23 ± 0.03	1.32 ± 0.02¶	1.25 ± 0.02
350	1.48 ± 0.02$^{¶¥\#}$	1.34 ± 0.05$^{¥\#}$	1.34 ± 0.04$^{¶\#}$	1.14 ± 0.03	1.20 ± 0.02	1.17 ± 0.02

Statistics: ¶significantly different from the same area (GA muscle) in the *wt* of the same group (unpaired two-tailed *t*-test, p<0.01); $^{¥}$significantly different from the corresponding value in Group B; $^{\#}$ significantly different from the corresponding value in Group C.

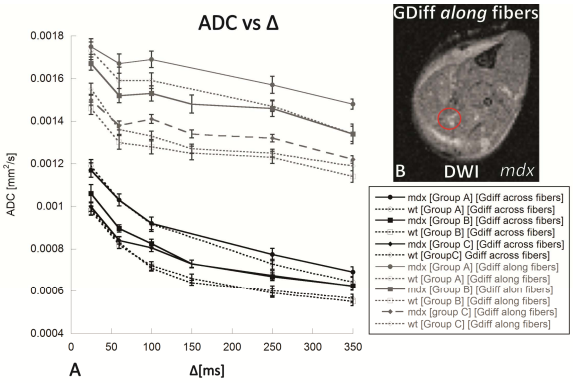

Fig1. A: ADC values of the GA muscle in *mdx* and *wt* mice, for each group of age (A, B, C), displayed as a function of Δ. Values were determined with Gdiff along (upper graphs) and across (lower graphs) the fiber direction. B: DWI axial image of *mdx* mouse hindlimb showing the ROI.

DISCUSSION

This study demonstrates the ability of diffusion MRI to discriminate dystrophic and healthy muscles especially when investigated using long diffusion times which enable muscle structure to be properly inferred in regime of restricted diffusion. Moreover, differences in diffusion properties of hindlimbs muscles in mice of different ages (7.5 vs 22 weeks) highlighted microstructural changes in GA muscle related to mouse age. These differences are consistent with a decreased water content in older mice with relative changes in size of muscle fibers as also confirmed by histology.

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