Quantitative NMRI and NMRS indices identify augmented disease progression after loss of ambulation in forearms of boys with Duchenne Muscle Dystrophy

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TARGET AUDIENCE: Researchers and clinicians with interest in neuromuscular diseases and biomarkers for therapeutic trials.

PURPOSE:. Duchenne muscular dystrophy (DMD), the most common of the muscle wasting disorders, (~1:3500 male births), leads to progressive muscle destruction resulting in loss of ambulation by teenage years. Replacement therapy for the missing dystrophin protein has begun in DMD boys and in the GRMD canine model of the disease. Prior studies in the GRMD canine model showed that quantitative NMRI and ^{31}P NMRS could provide multiple indices which graded with therapeutic dose in dogs[1].

Before using such indices to evaluate therapy in children, evolution of the natural course of DMD needed to be established. Since upper limbs were to be targeted in a future trial, NMRS and NMRI indices were collected specifically to charaterize disease progression in forearms of 24 DMD boys amenable to exon 53 skipping therapy.

SUBJECTS AND METHODS

The 24 DMD boys, (6-18 yrs, 14 non ambulant, exon deletions around exon 53) were enrolled in an observational evaluation protocol, and examined in a 3T-60cm Siemens Magnetom TRIO system, with the arm resting along the body. When the arm was retracted, the boy's body was inclined to align forearm with Bo. The two arms were examined in separate sessions on the same day, to be repeated at one year intervals.
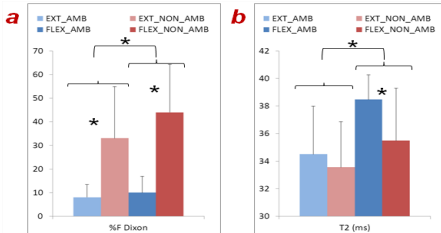
Each session comprised 3 items, each lasting ~20 min:

- NMRI multi-slice multi-echo measurement of T2 of muscle water in 3 10 mm slices in the forearm (17 echoes, TEs 8.7 to 147.9 ms,TR 4s, FOV 104x128 mm²)
- NMRI measurement of fat fraction by 3D, 3-point Dixon imaging (TE,2.75 3.95/5.15 ms) covering the same regions TR= 10ms, 166x480x128 mm3 with a pixel resolution of 1x1x1 mm3.
- NMRS of phosphate metabolites using an 11cm diameter surface coil predominantly facing flexor muscles (non localized, 500us hard pulse excitation, (TR=4s, NA=64 to 128, BW=3000 Hz, 2048 points)

Twelve indices were analysed:

- 2 NMRI indices: muscle water T2, deconvoluted from fat[2] and %fat signal (%F), were analysed in two regions of interest comprising flexor (FLEX) and extensor (EXT) muscles of the forearm (Fig1)
- 10 NMRS indices: 8 metabolic ratios combining: adenosine triphosphate (ATP), phosphocreatine (PCr), phospho mono- and di-esters (PME, PDE) and two pools of inorganic phosphate: cytosolic (Pi_a) and an anomalous alkaline pool present in dystrophic muscle (Pi_b)[3]; and corresponding pH values for both Pi pools (Fig1).

Fig. 1. Dixon map showing %F in FLEX and EXT ROIS and ^{31}P NMRS in right forearms of 2 DMD patients

RESULTS

Fig. 2. (a) Fat signal and **(b)** T2 values in flexor and extensor muscle groups of ambulant and non-ambulant patients

T2 were above normal in 15% of cases, %F exceeded 10% of signal in 70% cases and correlated to patient age (r=.58), as did 7 of 8 metabolic ratios. Flexor muscles had higher fat infiltration and higher T2 than extensor muscles, but T2 was reduced in muscles of non ambulant patients (Fig. 2.). All NMRS metabolic ratios correlated strongly to %F (R2=.11 to .65), p<0.01, but not to pH, nor to T2 values.

Most strikingly, %F and metabolic ratios **were highly related to years since loss of ambulation**) (p<0.05), whereas they evolved much less or not at all before. For all these indices, **the slope of progression in arms changed with loss of ambulation** (Fig3).
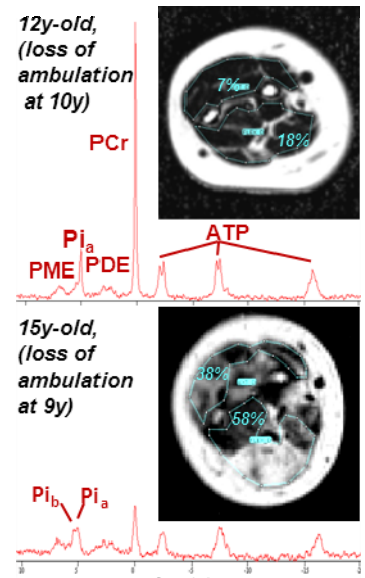
DISCUSSION AND CONCLUSION

Quantitative NMRI and NMRS provided a variety of indices which were abnormal in forearms of DMD patients with deletions around exon 53. Indices covered a broad range of fat infiltration and metabolic abnormalities which graded together and overall with patient age. NMRI indices showed greater involvement in flexor compared to extensor muscle groups, however, while fat infiltration progressed in non-ambulant patients, T2 was reduced, possibly indicating less inflammatory processes at later disease stages[5]. Fat infiltration and metabolic indices measured in arms of patients evolved remarkably differently before and after loss of ambulation. This might evoke concomittant progression in lower and upper limbs, or could relate to individual rate of disease progression which is reflected by age of loss of ambulation. Thus, PCr/ATP increase before loss of ambulation could reflect normal progression of muscle maturation in children[4] whilst PCr/ATP decrease after loss of ambulation would be expected from loss of metabolically active muscle. Taking such effects into account will improve design of outcome measures in trials.

REFERENCES [1] Thibaud, et al. Neuromusc.Disord. 2012, 22: 859; [2] Azzabou, et al. 20th ISMRM, 2012: 3429; [3] Wary, et al. NMR Biomed. 2012, 25: 1160-9; [4] Taylor DJ, et al. Mol Cell Biochem .1997, 174: 321-4; [5] Rooney W, et al. 21st ISMRM, 2013:689

Fig. 3. Dependance of **(a)** Fat signal fraction **(b)** PCr/ATP, **(c)** PDE/ATP and **(d)** proportion of alkaline Pi on age in ambulant patients **(Left)** and on years since loss of ambulation in wheelchair bound patients **(Right)**