MAGNETIC RESONANCE IMAGING IN DUCHENNE MUSCULAR DYSTROPHY: LONGITUDINAL ASSESSMENT OF NATURAL HISTORY OVER 18 MONTHS

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ABSTRACT: Introduction: In Duchenne muscular dystrophy (DMD), fat replacement of muscle may be a useful endpoint in trials of therapy, although progression in different muscle groups is uneven. In this study we assessed the progression of fat replacement with T1-weighted imaging over 2 9-month periods. Methods: Eight ambulant, corticosteroid-treated boys with DMD were imaged at 3 Tesla at 3 time-points (baseline and 9 and 18 months) with T1-weighted imaging to measure fat replacement. Results: The greatest increase in fat content was measured in the biceps femoris long head, vastus lateralis, and rectus femoris, whereas the biceps femoris short head and gluteus maximus progressed more slowly. None of the lower leg muscles studied changed significantly. Conclusions: MRI can measure specific changes in fat replacement of muscle over time, demonstrating the variability in rates of natural progression between muscle groups and identifying those muscles suitable for use as biomarkers in clinical trials.


Duchenne muscular dystrophy (DMD) is an X-linked recessive disorder. It has an incidence of around 1 in 3300 live male births,1 and is caused by mutations in the gene coding for dystrophin.2 The loss of dystrophin results in sarcolemmal fragility3 with muscle fiber necrosis, inflammation, and replacement of muscle by fat and connective tissue. Initially, the pelvic- and shoulder-girdle muscles are affected, and eventually there is loss of ambulation and death from respiratory and cardiac complications. The only current therapy is oral corticosteroid treatment,4 and with good management, affected individuals may survive into their late 20s.5

Several therapeutic options are being advanced for clinical trials.6,7 To prove efficacy there is a need for quantitative, non-invasive methods to assess disease progression. The standard clinical tests used for trials are dependent on patient cooperation and motivation (6-minute walk distance and myometric force testing) or are invasive and highly localized (muscle biopsy) and may not demonstrate sensitive changes over the short course of a clinical trial. Magnetic resonance imaging (MRI) provides a non-invasive means of evaluating the progression of individual muscle groups. Although there have been cross-sectional studies of involvement,8–10 this work identifies those muscle groups that will progress longitudinally over the short time course of a trial of 9 or 18 months for use as biomarkers.

METHODS

Participants. Approval from the local research ethics committee was obtained, and this study was in compliance with the Declaration of Helsinki. Eleven boys with a molecular diagnosis of DMD were recruited; 8 boys completed all 3 visits (age range 6.6–9.9 years, mean 8.7 years). The families were approached at a routine clinic visit, and informed consent was obtained from the parents after further discussion of the study. All boys were ambulant without the need for orthoses and were on corticosteroid therapy (mean duration 30.6 months) commenced at a dose of 0.75 mg/kg/day prednisolone as per international guidelines.4,11

MR Protocol. All scans were performed on a 3-T scanner (Philips Achieva; Philips, Best, The Netherlands) using an in-built body coil for transmission and reception. This avoided disturbing the child during the examination for surface coil repositioning in order to increase compliance. Images were collected at baseline and after periods of 9 and 18 months. Axial T1-weighted images of the musculature from ankles to iliac crest were obtained using a turbo spin echo sequence (TR/
TE/NSA = 671 ms/10 ms/2, TSE factor 3) with a slice thickness of 5 mm and gap of 10 mm, and a 256 × 192 matrix interpolated to 512 × 384. A field of view (FOV) of 380 mm and 3 stacks of 16 slices were used with a duration 15 minutes.

**Image Analysis.** Images were analyzed using MRIcro to draw regions of interest (ROIs) outlining muscles at mid-lower leg, thigh, and pelvis, using anatomical landmarks. Muscles were selected from anterior and posterior compartments in the lower leg (tibialis anterior, medial and lateral gastrocnemii) and thigh (rectus femoris, vastus lateralis, biceps femoris long and short heads, and gracilis). For the pelvis, the gluteus maximus was selected. Conservative ROIs were drawn to avoid areas of chemical shift artifact (Fig. 1). Muscle signal intensity (SI) was referenced to the subjects’ bone marrow intensity in the same image section (mean of left and right bone marrow) and expressed as a percentage relative to bone marrow intensity. The percentage changes were measured at baseline, 9 months, and 18 months.

**FIGURE 1.** Top left: T1-weighted images indicating regions of interest studied in the lower leg (top) and thigh (bottom). Top right: Longitudinal signal intensity of specified muscles on T1-weighted imaging compared with bone marrow intensity at baseline and 9-month and 18-month follow-up. Bars show median values for the group, and error bars show half the interquartile range. Brackets indicate significant differences (**P < 0.01, *P < 0.02 by Wilcoxon signed-rank test). Bottom: Progression of fat replacement in the thigh muscles over an 18-month period for 2 of the DMD children (left and right: top row = baseline; middle row = 9 months; bottom = 18 months). Figures indicate the signal intensity in the biceps femoris long head relative to bone marrow for that individual. Central figures indicate the mean for the group. The child on the right has a much faster rate of progression. Gracilis and biceps femoris are less affected. TA, tibialis anterior; LG, lateral gastrocnemius; MG, medial gastrocnemius; VL, vastus lateralis; RF, rectus femoris; Grac, gracilis; BFSH, biceps femoris short head; BFLH, biceps femoris long head; GMax, gluteus maximus (not shown).
percentage of the bone marrow SI. All ROIs used in the analysis were drawn by 1 investigator. An assessment of interobserver variation was made in previous work and was found to be extremely small (<0.3%).

Statistical Analysis. Analysis was performed using SPSS, version 17.0 (SPSS, Inc., Chicago, Illinois). Longitudinal comparisons were performed using a Wilcoxon signed-rank test and correlations with the Spearman rank. The null hypothesis was rejected for 2-tailed $P<0.05$.

RESULTS

The quantified signal intensities with respect to bone marrow for the 9 muscle groups analyzed are given in Figure 1 (top right). The muscles of the pelvis and thigh showed greater progression of fat replacement than the lower leg. The biceps femoris long head, vastus lateralis, and rectus femoris showed the greatest increase in fat content across the 18-month period (Fig. 1, bottom). The biceps femoris short head and gluteus maximus progressed significantly but to a lesser degree, yet the change was still significant. None of the muscles of the lower leg studied (tibialis anterior, medial and lateral gastrocnemius) changed significantly across the 18 months, with the smallest change in tibialis anterior. The magnitude of progression of fat replacement over 18 months did not correlate with age on entry to the trial for any muscle.

DISCUSSION

This longitudinal study of fat replacement in DMD boys demonstrated the use of non-invasive MRI to measure objectively the progression of individual muscle involvement over 18 months. The key findings are that the muscles of the pelvis and thigh showed greater progression of fat replacement than the lower leg. It was found that the biceps femoris long head, vastus lateralis, and rectus femoris showed the greatest progression and would be suitable targets for longitudinal assessment in clinical trials to examine the effects of therapies. From our knowledge that the spared gracilis signal intensity is approximately 32% of that of bone marrow (Fig. 1), we could estimate the median additional fat replacement in these 3 muscles to be $\sim32\%$, $\sim25\%$, and $\sim24\%$, respectively, across 18 months.

Assessing fat replacement by quantifying signal intensity with $T_1$-weighted imaging is subject to $B_1$ inhomogeneity, but we minimized these effects by careful subject placement and a consistent choice of reference within the tissue (the bone marrow). Our present and future studies of muscular dystrophy use the 3-point Dixon method, which avoids $B_1$ and $B_0$ inhomogeneity issues. However, these results clearly differentiate between those muscle groups with significant progression across a 9- or 18-month period (biceps femoris long head, rectus femoris, and vastus lateralis) and those with low (gastrocnemii) or no progression across the period (tibialis anterior, gracilis). In the latter muscles, $T_2$-relaxation time measurements may provide information about edema and infiltration preceding fat replacement, but these measurements were not performed in the present study. The assessment of muscle hypertrophy may also be useful before fat replacement occurs.

We have shown that MRI can be used non-invasively to assess the natural progression of pathology in DMD patients on steroid therapy and we have identified those muscle groups whose fat replacement might be measured as a biomarker in a longitudinal trial of therapy. This provides the opportunity to measure the early effects of therapy on specific muscle groups, which may be detectable by MRI before standard clinical tests.

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REFERENCES