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T₂ mapping provides multiple approaches to characterize muscle involvement in neuromuscular diseases: a cross-sectional study of lower leg muscles in 5–15 year old boys with Duchenne Muscular Dystrophy

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Abstract

Purpose—Skeletal muscles of children with Duchenne muscular dystrophy (DMD) have enhanced susceptibility to damage and progressive lipid infiltration, which contribute to an increase in magnetic resonance proton transverse relaxation time (T₂). Therefore, examining T₂ changes in individual muscles may be useful for monitoring disease progression in DMD. In this study we utilized mean T₂, percent elevated pixels, and T₂ heterogeneity to assess changes in composition of dystrophic muscles. In addition, we used fat saturation (fatsat) to distinguish T₂ changes due to edema and inflammation from fat infiltration in muscles.

Methods—Thirty subjects with DMD and 15 age-matched controls underwent T₂-weighted imaging of their lower leg using 3-T MR system. T₂ maps were developed and four lower leg muscles were manually traced (soleus, medial gastrocnemius, peroneal and tibialis anterior). Mean T₂ of the traced regions of interest (ROI), width of T₂ histograms, and percent-elevated pixels were calculated.

Results—We found that even in young children with DMD, muscles had elevated mean T₂, were more heterogeneous, and had a greater percent-elevated pixels in the lower leg muscles than controls. T₂ measures decreased with fat saturation, but were still higher (p<0.05) in dystrophic muscles than controls. Further, T₂ measures showed positive correlations with timed functional tests (r=0.23–0.79).

Conclusion—The elevated T₂ measures with and without fat saturation in all ages of DMD examined (5–15 years) compared to unaffected controls indicate that the dystrophic muscles have increased regions of damage, edema, and fat infiltration. This study shows that T₂ mapping provides multiple approaches that can be effectively utilized to characterize muscle tissue in children with DMD even in the early stages of the disease. Therefore, T₂ mapping may prove clinically useful in monitoring muscle changes due to disease process or therapeutic interventions in DMD.

Keywords

Duchenne muscular dystrophy; skeletal muscle; MRI; proton transverse relaxation time; T_2 mapping; heterogeneity; biomarker

Introduction

Duchenne muscular dystrophy (DMD) is one of the most common inherited, degenerative neuromuscular disorders characterized by early and progressive involvement of muscles. DMD is an X-linked recessive disease affecting 1 in 3500 male births (1). The genetic cause of DMD has been known since 1987 (2), but there is still no cure for the disease. Although many therapeutic strategies have been shown to be successful in animal models, their effectiveness in clinical trials has only begun to be evaluated (3). Outcome measures in this area of research have been largely limited to muscle biopsies and measures of muscle strength and functional ability. Unfortunately, muscle biopsies are invasive and may not be truly representative of the response of the entire muscle and other muscles in dystrophy (4). Thus, there is a need for non-invasive biomarkers that can provide global information of changes in composition of muscles due to disease progression. Strength and functional tests are important clinical tools for monitoring the progression of disease in working muscle groups and understanding the pathology of disease (5). However, these tests are dependent on subject motivation and compliance, which can be particularly challenging in young children. Further, functional testing is limited during the later stages of disease when the children with DMD lose the ability to walk. Therefore, adopting additional outcome measures may be valuable to study the disease process and the effectiveness of therapeutic interventions in DMD.

Magnetic resonance imaging (MRI) has become an important non-invasive tool for studying muscle structure and composition over the last two decades and has been successfully used to examine disease status in children with DMD (4,6–17). One potential quantitative approach to study skeletal muscle in muscular dystrophies is utilizing proton transverse relaxation time (T_2), and elevated T_2 has previously been observed in dystrophic skeletal muscles (12,18). T_2 distribution within dystrophic muscles has also been examined (18), and investigators from a recent study reported changes in the distribution of T_2 in thigh muscles in response to corticosteroid therapy (13). In another study by Wansapura et al. (15), the myocardium in DMD was examined, and heterogeneity in the left ventricle as measured by distribution of T_2 was found to be more sensitive to the early dystrophic changes in comparison to mean T_2 . The authors of the study suggested that the assessment of tissue heterogeneity (as opposed to the examination of mean T_2 values) may provide a greater sensitivity in detecting early changes in dystrophic muscles. Collectively, the results show considerable promise of using T_2 as a measure of muscle involvement in DMD.

In this study we performed a comprehensive evaluation of various methods of T_2 mapping to characterize disease involvement and heterogeneity in several lower leg muscles of boys with DMD over a wide range of ages. Examining the lower leg may have an advantage over more proximal muscles, as the disease progression tends to be slower in the lower leg muscles and therefore may be more applicable to use as outcome measure over a greater range of ages. Furthermore, while T_2 may be sensitive to disease progression, the interpretation of patho-physiological changes resulting in the T_2 change is often challenging as T_2 can be influenced by a number of factors, including fat infiltration, inflammation and edema associated with muscle damage (19–23). One approach to provide further insight into the disease process is to also implement fat suppression with T_2 -weighted images. An elevated T_2 in fat saturated images in DMD relative to controls may indicate the presence of

changes occurring from inflammation or edema. Therefore, the approach of using both non-fat saturated (non-fatsat) and fat saturated (fatsat) T₂ MRI may help to elucidate the specific changes observed in T₂ of muscles in DMD.

Overall, the aims of this study were to: 1) compare non-fatsat and fatsat T₂-based MRI measures (mean T₂, percentage of affected area and heterogeneity) in lower leg muscles of children with DMD to controls; 2) evaluate the T₂-based MRI measures across different age groups in DMD and compare those to healthy controls; and 3) establish the relationships between T₂-based MRI measures and clinical functional tests.

Material and methods

Subjects

In this cross-sectional study, 30 boys with DMD (5–15 yrs) and 15 healthy boys of similar age participated in MRI and functional testing. DMD was confirmed by muscle biopsies and/or genetic testing. Demographic information of all the participants is shown in Table 1. The study was approved by the Institutional Review Board of the University of Florida. After complete description of the study, informed written consent was obtained from the parents/guardian, and each subject provided his written assent.

MR Acquisition

MR procedures were performed using a 3.0T whole body MRI scanner (Philips Achieva Quasar Dual 3T) at the McKnight Brain Institute of the University of Florida. In order to obtain valid measurements for the study, the subjects were instructed to avoid any excessive physical activity beyond their normal activities for two days before MRI. Further, they were asked to use a wheelchair or similar mobility device while traveling (to the university or in airports) to avoid excessive walking. MR scanning of the right lower leg was performed using a SENSE 8 channel knee volume coil [Field of view (FOV): 12–24 × 12–14 cm²] with the subjects in a supine position without the use of sedation. Padded supports were used to help maintain the leg in a fixed position. The coil was placed around the proximal 1/2 of the lower leg (measured from calcaneus to tibial plateau) to capture the maximum cross-sectional area (CSA max) of the triceps surae, peroneus, and tibialis anterior muscle. T₂-weighted SE images [12–18 axial slices; Slice thickness= 7mm; Repetition time (TR)=3s; echo times (TE's): 5 echoes evenly spaced between 20–100 ms] with saturation [Spectral presaturation with inversion recovery (SPIR)] and without fat saturation were acquired with a refocusing angle of 120 degrees. Fat-sat data was collected for 10 healthy subjects; although, in two muscles (Peroneal and MG) of two healthy subjects, there appeared to be artifacts in images and therefore those two data points were not included for further data analysis in the comparisons. During data collection, each subject was shown a movie of his choice using an in-magnet video display system in order to facilitate compliance and to decrease the likelihood of any movement during scanning. One parent/guardian and one staff member remained with the child in the MR room for the duration of the scan.

Functional Testing

After MR testing, each subject performed three timed functional tests that have previously been used in ambulatory boys with DMD to assess lower extremity function (4,24–28). Out of 15 controls, we collected functional data on 14 boys, as one subject did not participate in functional testing due to time constraints. The timed functional tasks included walking 30 feet (30-ft walk), climbing four steps (climb stairs) and rising from the floor (supine to stand). Each test was performed three times. Simple standardized instructions were provided to the subjects, and the time required to complete each task was recorded using a stopwatch. The fastest trial for each test was used for further analysis. Out of thirty DMD subjects, four

subjects did not complete the supine to stand activity. Among those four, three did not climb stairs and two could not participate in any of the functional tests, as they were non-ambulatory. In addition to timed functional tests, each subject was also scored on Brooke Lower Extremity Functional Scale (24).

MRI Data Analysis

T_2 mapping was performed by using the axial slices of the lower leg in the region of the largest cross sectional area (CSA) of the lower leg. To improve the coverage of the mid-calf region and to increase reliability, three consecutive slices were chosen for data analysis. The chosen slices had a large representation of all the muscles of interest, were around the max CSA of the lower leg (typically the middle slices) and were in the region that corresponded with the most proximal slice at which both the flexor digitorum longus and popliteal muscles were visually present. Pixel by pixel T_2 maps were generated for the corresponding slices by applying a mono exponential decay model to four echo times (TE's: 40, 60, 80, and 100 ms) using a custom written IDL software. Due to the possibility of stimulated echoes, the first echo point (20 ms) was not included in the analysis to obtain a more accurate reflection of the primary T_2 decay (29). Four calf muscles (soleus: SOL; medial gastrocnemius: MG; tibialis anterior: TA, and peroneal: PER) were manually traced on the obtained T_2 maps with care to avoid the inclusion of any subcutaneous fat. For both PER and TA, the fascia that divides the muscles into compartments, was excluded (Fig.1). Preliminary analysis showed that the TA and PER were significantly more heterogeneous than other leg muscles in control as well as DMD groups. We speculated that some of this heterogeneity might be attributed to presence of fascia within these muscles. Therefore, we tested the effect of exclusion of the fascia that separates the different compartments within PER and TA on muscle heterogeneity. We found that though exclusion of fascia did not affect mean T_2 of both muscles (PER: 50.8 vs. 50.3; TA: 45.5 vs. 46.0 ms), yet this resulted in a reduced heterogeneity of the muscles (PER: 15.2 vs. 10.0; TA: 13.5 vs. 8.6 ms). The heterogeneity of the muscles was quantified by measuring the widths of T_2 histograms (further explanation on the technique is provided later in the text). Since our main variable of interest was the muscle tissue itself and not the fascia, the analysis without the fascia were used for the main comparisons in this study. The non-fat and fat sat images were processed separately but since the images were acquired with the similar geometry (i.e. FOV and number of slices), the same regions of interest (ROI's) were used in non-fatsat and fatsat images.

For every muscle, T_2 values for each pixel within the traced region of interest of the three slices were added and plotted as histograms. The mean T_2 values for individual muscles were then obtained. We further quantified the relative area of involvement in children with DMD by using an objective thresholding technique. For each muscle, the control histograms (from 10 healthy subjects; who had both non-fatsat and fatsat data) were added to obtain a normalized histogram for that particular muscle and the 95th percentile of the normalized histograms was used as threshold (Fig.1). We determined the optimal threshold for measuring elevated T_2 values in dystrophic muscle by comparing the thresholds set at various levels between 90 and 100 percentiles of histogram values derived from control subjects. It was determined that setting the threshold at the 95% percentile was optimal as the higher values tended not to capture the minimally involved subjects, while the lower values would tend to result in 100% of the pixels being above the threshold in more involved muscles and therefore would not be capable of monitoring the disease progression. Importantly, this threshold value was determined on a pixel-by-pixel basis on the accumulated histograms of controls rather than on the mean T_2 values of each control subject. This choice was based on the observation that heterogeneity of individual control muscles was greater than the heterogeneity of mean T_2 values of the population. We also

used a percentile rather than a SD (e.g., 2SD) since there was no physiological rationale for the distribution in diseased muscle to be normal. The threshold T_2 values were determined separately for both fatsat and non-fatsat images in each muscle of interest (Non-fatsat: SOL=48.0ms, MG=46.0ms, PER=49.0ms; TA: 44.0 & Fatsat: SOL= 46.0ms, MG=43.5ms, PER=44.0ms, TA=42.5ms). Pixels with elevated T_2 values above the threshold (95th percentile) in the muscles of interest were considered elevated and were expressed as a percentage (%) of total pixels.

In addition, the heterogeneity of the muscle tissue was quantified by examining the distribution of T_2 within the muscles. The width of the histograms was measured by full width at half maximum (FWHM) and full width at quarter maximum (FWQM) as shown in figure 1.

Statistical analysis

A non-parametric test (Mann-Witney U test) was performed to compare mean T_2 , % of elevated pixels above threshold, FWHM, and FWQM across two groups for both non-fatsat and fatsat data using IBM SPSS Statistics 20 software. Within-group comparisons were made using Wilcoxon (two-related sample) test and Bonferroni correction was used for multiple comparisons. The significance (2-tailed) p values were reported for all the comparisons. For functional data analysis, the DMD subjects who were unable to perform any functional test were given the highest score in that activity. Therefore, Spearman's rank correlation was performed to compare T_2 -based MRI measures and functional tests. The level of significance was set at p 0.05.

Results

T_2 -based MRI measures

Non-fatsat mean T_2 of all four lower leg muscles was significantly higher (p 0.001) in DMD subjects in comparison to controls (Table 2; Fig.1). As expected, mean T_2 values decreased with fat saturation, and fatsat mean T_2 was significantly elevated (p 0.001) in dystrophic muscles as compared to controls. On average, the non-fatsat T_2 was 10 % higher than fatsat T_2 in DMD subjects (two fold greater than the % change in controls). Among DMD subjects, TA had significantly lower mean T_2 than other muscles in both non-fatsat and fatsat images (p 0.001).

The affected muscle area was quantified by using a thresholding technique and was represented by the percentage of elevated T_2 pixels above the threshold value for the muscle in unaffected healthy subjects. The percentage of affected area was significantly higher for all muscles in DMD subjects compared to controls in both non-fatsat and fatsat data (p 0.001; Fig. 2). A large variability was observed in the percent-elevated pixels within dystrophic muscles (range: 5–100%), with the TA muscle showing a trend towards being less affected than SOL and MG.

The heterogeneity in the muscles was determined by measuring the width (FWHM and FWQM) of the T_2 histograms. The muscles in DMD subjects were found to be more heterogeneous in composition in comparison to controls (Table 3). For all muscles tested, the histograms of DMD subjects were shifted right and demonstrated increased widths in comparison to controls (SOL= MG> PER>TA, p 0.01). After fat suppression, heterogeneity in dystrophic muscles decreased by, on average, 30%, and dystrophic SOL and MG muscles did not show any significant difference from controls. Within dystrophic muscles, TA was significantly less heterogeneous than other muscles among DMD subjects (p 0.001).

Differences in T₂-based MRI measures across age groups

The children with DMD were divided into three groups: 5–7.9 yrs (n=12), 8–11.9 yrs (n=14), and 12–15 yrs (n=4). For analyzing age-related differences in T₂-based MRI measures, DMD subjects in each age group were compared to healthy controls. Mean T₂ and percent-elevated pixels were significantly higher ($p < 0.01$) for muscles in DMD in all age groups (Fig. 4). Among measures of heterogeneity, FWQM was relatively more sensitive than FWHM for eliciting differences between dystrophic and control muscles as well as within dystrophic muscles across different age groups. Overall, the dystrophic muscles were more heterogeneous than controls in all age groups in non-fatsat images. However, the differences between dystrophic and control muscles in terms of heterogeneity were no longer observed in the majority of muscles examined after fat saturation (Table 4). Within children with DMD, the TA muscle had significantly lower mean T₂ and heterogeneity than other lower leg muscles in younger age groups in both non-fatsat and fatsat images (5–8 yrs & 8–12 yrs, $p < 0.05$).

MR measures and Functional tests

T₂ measures obtained from non-fatsat images had better correlations with functional measures in comparison to fatsat images. Among functional tests, Brooke score and supine up test had the strongest correlation with T₂-based MRI measures for all muscles (Table 5, Fig.3). In addition, among measures of heterogeneity, FWQM was found to be, on average, stronger correlated to functional measures in comparison to FWHM (Table 5).

Discussion

In this study we exploited T₂ measures on a pixel-by-pixel basis to examine the extent of involvement in lower leg muscles of children with DMD. We utilized analysis techniques beyond conventional mean T₂ measures to evaluate the percentage of the affected region and heterogeneity within and among muscles. Our main findings were: 1) T₂ values were elevated in lower leg muscles of children with DMD in comparison to controls in all age groups examined, including young subjects (5–8 years); 2) T₂ was also elevated in DMD with fat saturation applied, indicating factors other than increase in lipid content contribute to elevated T₂; 3) using T₂ histograms with a thresholding technique to estimate the percentage of affected muscle and measuring the FWHM/FWQM as an index of muscle heterogeneity provide additional measures of monitoring disease involvement in children with DMD; and 4) T₂ measures, including alternate T₂ based approaches (i.e., percent elevated pixels and heterogeneity), were strongly correlated to functional abilities in this population.

Multiple muscles and age groups

This study evaluated involvement of multiple muscles of the lower leg in children with DMD using various T₂-based approaches. The advancement of disease is typically slower in the lower leg muscles relative to the upper leg in DMD. Therefore, we focused on the lower leg muscles in this study as these muscles may provide a greater window for monitoring disease progression and treatment throughout the lifespan of children with DMD. Our results showed that T₂ was elevated in the SOL, MG, PER, and TA muscles compared to age-matched controls. We found considerable variability in terms of muscle involvement among children with DMD within the same age groups. Overall, there was a trend of progressive increase in non-fatsat T₂-based MRI measures in the dystrophic muscles across age groups (Fig. 4). Importantly, even the young age group (age 5–8 years) showed significantly elevated T₂ measures in dystrophic muscles in comparison to controls. Therefore, T₂ may have an advantage compared to other outcome measures typically utilized to measure disease progression in DMD in which disease progression is not observed until older ages

(e.g., 6-min walk test) (5). The observation of elevation in mean T_2 has been previously reported in the TA (18) and thigh muscles (12) of children with DMD. The present study extends these findings by showing differences among the lower leg muscles, including that the TA had lower mean T_2 values than SOL, MG and PER, particularly in younger age groups in DMD. Therefore, the TA may be relatively preserved muscle in the early stages of the disease. Our findings are different from the results of an earlier study in DMD, which showed the loss of dorsiflexor (DF) muscle strength before plantarflexors (PF) via manual muscle strength testing (30). However, our results are supported by recent imaging studies (CT/ MR), which also found relative sparing of TA compared to other muscles of lower leg (16,17,31). Furthermore, a previous study from our laboratory on the age related differences in cross-sectional area and strength measures showed that the specific torque of the PF muscles was impaired four fold in the boys with DMD compared to healthy subjects, whereas, the deficit in specific torque of dystrophic DF was only two fold lower compared control muscles (14). Therefore, this study also supports the relative sparing of the TA muscle in Duchenne muscular dystrophy. Similarly, selective sparing of some muscles (gracilis, sartorius, and semitendinosus) has been observed in the upper leg also (4,6,8,11,12). However, the etiology for this selective muscle involvement and relative sparing in the lower extremities of boys with DMD is still unclear. One possibility is that the muscles relatively spared may undergo reduced loading during eccentric contractions with daily activities. Recent studies on quantitative assessment of gait have reported altered gait patterns in children with DMD (32–35). The altered patterns of lower limb loading during walking may influence the progression of muscle pathology in this population. Therefore, a detailed analysis of kinetics and kinematics of gait concentrating on the muscle activity and types of contractions during various phases is recommended for future studies. Considering the variations in responses of individual muscles to the disease, it is important to use caution when interpreting the overall status of the disease from a single muscle analysis in DMD.

Fat saturation

Mean T_2 was significantly higher in dystrophic muscle in comparison to controls, and these differences between groups were observed even after fat saturation. Therefore, these results support the notion that factors other than lipid infiltration, i.e. edema and inflammation contribute to elevated T_2 in dystrophy (20). Furthermore, we found that the percent decrease in these T_2 based MR measures after fat saturation was much greater in muscles of older children with DMD than younger ones. Also, fatsat T_2 values tended to increase a smaller amount with an increase in the age categories in comparison to non-fat T_2 values. These results confirm that with progression of disease, lipid infiltration increases in DMD.

Interestingly, after fat saturation, the TA muscle showed a trend towards progressive decrease in T_2 based MRI measures across increasing age groups in contrary to other muscles (Fig. 4). This may be due to less edematous changes with age or increase in fibrotic tissue in the older children with DMD. Notably, PER had comparatively higher threshold than other muscles in healthy controls in non-fat suppressed images (50 ms) but the threshold for PER muscle dropped by 6 ms (44 ms) in fat suppressed images. These results reflect that the composition of even healthy PER muscles may be different from other muscles of lower leg with the presence of more fat.

It should be noted that although fat saturation effectively eliminated the majority of lipid signal from the images, it typically does not completely remove fat contribution homogeneously. Therefore, one may argue the possibility of contribution of fat to the elevated T_2 values after fat saturation in DMD than controls. However, our results showed that even in young subjects when changes from fat infiltration are expected to be minimal, T_2 measures were still elevated in DMD than controls. Therefore, it appears that elevated T_2 in the fatsat data of DMD subjects was not simply due to incomplete fat saturation. Future

studies aimed at quantifying the contribution of lipid to the progression of disease in DMD using three-point Dixon technique may be valuable.

Alternate T_2 based MRI approaches

In this study, we showed that T_2 histograms provide alternate approaches to quantify muscle involvement in DMD. We used an objective thresholding technique for quantifying the percentage of affected area in dystrophic muscles. We found that in comparison to conventional method of comparing mean T_2 changes in muscles, the percent-elevated pixels provided a greater range of values for monitoring changes in dystrophic muscles (40–85 ms vs. 0–100%; Fig. 4). Therefore, we anticipate that the latter method may be more sensitive than mean T_2 measure for following the subsequent minor changes within the dystrophic muscles in response to disease process or therapeutic interventions. Phoenix et al. (36) utilized this technique of calculating a threshold T_2 value based on unaffected healthy muscle in limb girdle and Becker muscular dystrophy. In that study the area corresponding to pixel values above threshold was defined as cross-sectional area of fat (FCSA) in the lower leg muscles of adult dystrophy patients. However, that study did not consider that T_2 may also be elevated in dystrophic muscles due to inflammation, fluid accumulation, and shifts in water compartmentalization (37,38). Also, our methodology for calculation of threshold T_2 value was different from the previous study. In that study the authors pooled the data from the TA, SOL, and gastrocnemius muscles of three normal subjects to produce group mean signal intensity and used 95th percentile of normal muscle signal as common threshold value for quantification of FCSA in dystrophic muscles. However, considering the possibility of innate differences between muscles even in healthy subjects (as observed in TA and PER in this study), we calculated the threshold T_2 value for all the muscles separately. We found that PER had the highest threshold and the TA had the lowest threshold among chosen muscles of the lower leg. Therefore, if we had used the previous technique, we might have either overestimated the percentage of elevated pixels in dystrophic PER muscles or underestimated the affected area in other muscles, especially TA.

We also evaluated widths of T_2 histograms to quantify muscle heterogeneity in children with DMD. Previous studies have shown DMD to be characterized by fibrosis, lipid, and inflammation (11,38). Since edema/inflammation and fat infiltration have longer T_2 values than healthy muscle tissue and fibrosis lies on the opposite side of T_2 spectrum (i.e. shorter mean T_2), together these pathophysiological changes will result in greater distribution of T_2 within dystrophic muscles. The heterogeneity in dystrophic muscles had been previously reported by Wansapura et al. (15). The authors found that measuring heterogeneity with T_2 histograms was sensitive to early myocardial changes even when mean T_2 in DMD subjects remained unchanged. However, in our study, both mean T_2 and measures of heterogeneity were able to detect differences in the muscles of young children with DMD. Therefore, quantification of tissue heterogeneity may provide additional information about the change in composition of muscle tissue in DMD. Importantly, the assessment of T_2 distribution may be useful in the stages where muscle is replaced by fibrotic tissue, thereby nullifying any overall increase in mean T_2 from edema or fat infiltration. Further, we introduced FWQM as an additional measure of heterogeneity in our study. FWQM was examined based on the observation that children with DMD often have a shoulder on the histogram resulting in broader distribution near the base of the histograms (Fig. 1e). Therefore, we hypothesized that FWQM may be more sensitive to distribution changes in comparison to FWHM. We found that FWQM had comparatively stronger correlations in MG, TA, and PER with various functional tests than FWHM and this measure of heterogeneity was relatively more sensitive than FWHM for extracting differences between dystrophic and control muscles as well as within dystrophic muscles across various age groups. Therefore, we recommend that

FWQM should be considered as an important measure of heterogeneity in future studies related to muscle dystrophies.

Our study also showed that mean T_2 and alternate T_2 based MR approaches were strongly related to the functional tests, specifically supine up test and Brooke score. These findings were in accordance with previous studies that showed a positive correlation between mean T_2 of the dystrophic muscles and clinical assessments, i.e. supine to stand, and 30 ft walk tests in DMD (12). This further strengthens the validity of using T_2 measures as an adjunct clinical tool for monitoring the changes in the individual muscles in this population.

Conclusion

The results of this study showed that T_2 mapping provides multiple approaches that can be effectively utilized to characterize muscle tissue in children with DMD using a combination of fat saturated and non-fat saturated images. This approach enabled measuring muscle involvement using mean T_2 of multiple muscles, examining percentage of affected muscle area, and providing an index of muscle heterogeneity. Therefore, we anticipate T_2 mapping can be used as a non-invasive and sensitive biomarker for quantification of early and subtle muscle changes due to disease process or therapeutic interventions in DMD. Future longitudinal studies with serial examinations of muscles of the lower leg would be valuable to extend the findings of the present study and provide further information on the potential of these T_2 analysis strategies to monitor disease progression in DMD.

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List of Abbreviations

CSA max	maximum cross-sectional area
DMD	Duchenne muscular dystrophy
Fatsat	fat saturation
FCSA	cross-sectional area of fat
FOV	field of view
FWHM	full width at half maximum
FWQM	full width at quarter maximum
MG	medial gastrocnemius
Non-fatsat	non-fat saturation
PER	peroneal
ROI	region of interest
SOL	soleus
SPIR	spectral presaturation with inversion recovery
TA	tibialis anterior

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Figure 1.

Top panel: a) Demonstration of manually traced muscles of lower leg on an MR image; b) T_2 map of the lower leg of a 10-year old control boy and; c) 11 year old boy with DMD. Note the higher T_2 values in the muscles of the boy with DMD in comparison to healthy control. Lower panel: Normalized T_2 histogram of SOL muscle from a control subject (d, e, black) and from a DMD subject (e, red). Widths of T_2 histogram are measured as the full width at half maximum (FWHM) and full width at quarter maximum (FWQM); a threshold T_2 value (e) of 48 (ms) is represented by the green line. Note the rightward shift of the DMD histogram (red); and a “shoulder” on the histogram representing broader distribution at base. The number of pixels above the threshold value is used to determine the percent-elevated pixels in the DMD SOL muscle.

Figure 2.

Comparison of percent elevated pixels between control and DMD subjects in non-fat sat data (a) and fatsat (b) data. Boxes represent 25 and 75 percentiles and arrow bars indicate 5 and 95 percentile with the median presented with a horizontal line within the DMD box plots. ** Significant difference between groups ($p < 0.001$).

Figure 3.

Example plots showing correlation of percent elevated T₂ pixels in the TA muscle with time to walk 30ft (a) and time to stand from supine (b). Subjects not able to complete the task are not included in the figure.

Figure 4.

Top panel: Comparison of Mean T₂ in PER (a) and TA (b) muscles between controls and children with DMD in different age groups. Lower panel: Changes in percent elevated pixels across different age groups in PER (c) and TA (d) in children with DMD in comparison to controls. Boxes represent 25 and 75 percentiles and arrow bars indicate 5 and 95 percentile with the median presented with a horizontal line within the DMD box plots. **Significantly different between groups (p 0.001); *Significantly different between groups (p 0.01); \$Significantly different between groups (p 0.05)

Table 1

Comparison of healthy and DMD group on various demographic features. Values are expressed as mean $\pm$ SD or as otherwise indicated.

Characteristic	Controls (n=15)	DMD (n=30)	P
Age (y)	9.7 $\pm$ 2.7	9.6 $\pm$ 2.5	0.873
Body Weight (kg)	32.8 $\pm$ 10.4 [#]	32.02 $\pm$ 12.4	0.833
Height (cm)	139.7 $\pm$ 15.7 [#]	125.9 $\pm$ 13.1	0.010 [*]
BMI (kg/m ²)	18.2 $\pm$ 7.1 [#]	19.7 $\pm$ 4.1	0.016 ^{\$}
Non-ambulatory/Ambulatory	0/15	2/30	NA
Brooke score (median)	1	2 (1–9)	NA

Abbreviation: NA, not applicable.

^{*} Significantly different between groups (p 0.01);

^{\$} Significantly different between groups (p 0.05);

[#] Information available for 14 subjects only.

Table 2

T₂ relaxation times (ms) for non-fatsat and fatsat images of control and DMD subjects.

Muscle	Non-fatsat		Fatsat	
	Control	DMD	Control	DMD
SOL	40.3 ± 1.1	50.6 ± 5.5 **	38.2 ± 0.8	45.3 ± 3.8 **
MG	39.2 ± 1.9	50.8 ± 7.9 **	37.1 ± 0.5	45.2 ± 4.3 **
PER	39.7 ± 1.5	51.2 ± 8.4 **	37.3 ± 0.6	44.2 ± 3.9 **
TA	38.7 ± 1.3	45.7 ± 5.0 **	37.3 ± 1.2	41.5 ± 1.6 **

Values are expressed as mean ± SD.

** Significantly different between groups (p 0.001)

Table 3

Comparison of FWHM (ms) and FWQM (ms) between control and DMD subjects.

Muscle	FWHM			FWQM		
	Non-fatsat	Fatsat		Non-Fatsat	Fatsat	
	Control	DMD		Control	DMD	
SOL	9.1 ± 1.3	11.3 ± 2.3 [*]	7.9 ± 1.1	13.8 ± 2.1	16.4 ± 3.4 [*]	11.9 ± 1.9
MG	8.4 ± 1.5	10.9 ± 2.8 [*]	7.3 ± 0.9	13.0 ± 2.4	16.8 ± 4.6 [*]	10.9 ± 2.0
PER	7.3 ± 1.1	9.7 ± 3.3 [*]	5.6 ± 0.8	11.7 ± 1.4	17.9 ± 6.5 ^{**}	8.4 ± 1.0
TA	7.0 ± 1.1	8.2 ± 2.0 [§]	5.5 ± 1.3	10.4 ± 1.4	14.3 ± 4.5 ^{**}	8.1 ± 1.7

^{**} Significantly different between groups (p 0.001);^{*} Significantly different between groups (p 0.01);[§] Significantly different between groups (p 0.05)

Table 4
Comparison of measures of heterogeneity (FWHM and FWQM) between control and DMD subjects in different age groups.

		Controls			DMD		
		5-15yrs	5-8yrs	8-12 yrs	12-15yrs		
FWHM	Non-fatsat	SOL	9.1 ± 1.3	10.2 ± 1.9	11.7 ± 2.6 [*]	13.0 ± 1.4 ^{**}	
		MG	8.4 ± 1.5	9.7 ± 1.9	10.9 ± 2.8 [§]	14.5 ± 1.8 ^{**}	
		PER	7.3 ± 1.1	8.0 ± 1.4	9.9 ± 3.2 [*]	13.9 ± 4.4 ^{**}	
		TA	7.0 ± 1.1	7.7 ± 0.9	8.5 ± 2.5	8.7 ± 2.5	
Fatsat		SOL	7.9 ± 1.1	8.9 ± 1.7	8.3 ± 1.8	9.1 ± 1.1	
		MG	7.3 ± 0.9	8.2 ± 1.5	8.6 ± 3.2	10.3 ± 2.6 [§]	
		PER	5.6 ± 0.8	6.6 ± 1.4	7.1 ± 1.7 [*]	8.7 ± 1.8 [*]	
		TA	5.5 ± 1.3	6.8 ± 1.0 [§]	6.2 ± 0.9	5.7 ± 0.8	
FWQM	Non-fatsat	SOL	13.8 ± 2.1	15.0 ± 2.7	16.8 ± 3.7 [§]	19.6 ± 1.2 ^{**}	
		MG	13.0 ± 2.4	15.2 ± 3.6	16.5 ± 4.5 [§]	22.6 ± 3.5 ^{**}	
		PER	11.7 ± 1.4	14.4 ± 3.2 [*]	18.6 ± 6.9 [*]	26.2 ± 5.0 ^{**}	
		TA	10.4 ± 1.4	12.2 ± 2.0 [*]	14.4 ± 4.6 [*]	20.3 ± 5.2 ^{**}	
Fatsat		SOL	11.9 ± 1.9	13.3 ± 2.7	12.6 ± 2.5	14.2 ± 3.4	
		MG	10.9 ± 2.0	12.4 ± 2.6	13.5 ± 5.6	14.7 ± 2.9 [§]	
		PER	8.4 ± 1.0	10.1 ± 2.5	10.8 ± 3.0 [§]	12.9 ± 3.2 [*]	
		TA	8.1 ± 1.7	10.2 ± 1.4 [*]	8.8 ± 1.4	8.8 ± 1.3	

Values are expressed as mean ± SD.

^{**} Significantly different between groups (p 0.001);

^{*} Significantly different between groups (p 0.01);

[§] Significantly different between groups (p 0.05).

Table 5

Comparison of T₂-based MRI measures to functional tasks. Correlation coefficients (r) for various comparisons are reported in the table. Subjects who were unable to perform any functional activity were given highest score for doing rank correlations.

	Mean T ₂				Elevated Pixels (%)				FWHM				FWQM			
	Sol	MG	PER	TA	Sol	MG	PER	TA	Sol	MG	PER	TA	Sol	MG	PER	TA
30-ft walk	0.42 [§]	0.57 [*]	0.61 [*]	0.59 [*]	0.43 [§]	0.49 [*]	0.55 [*]	0.62 [*]	0.32	0.42 [§]	0.33	0.23	0.31	0.51 [*]	0.58 [*]	0.55 [*]
Supine up	0.63 [*]	0.69 [*]	0.77 [*]	0.72 [*]	0.63 [*]	0.60 [*]	0.77 [*]	0.73 [*]	0.57 [*]	0.57 [*]	0.48 [*]	0.33	0.60 [*]	0.64 [*]	0.79 [*]	0.70 [*]
Stairs	0.52 [*]	0.64 [*]	0.72 [*]	0.63 [*]	0.52 [*]	0.54 [*]	0.71 [*]	0.65 [*]	0.51 [*]	0.58 [*]	0.45 [§]	0.33	0.51 [*]	0.67 [*]	0.73 [*]	0.68 [*]
Brooke score	0.62 [*]	0.64 [*]	0.75 [*]	0.70 [*]	0.60 [*]	0.59 [*]	0.73 [*]	0.67 [*]	0.58 [*]	0.59 [*]	0.43 [§]	0.44 [§]	0.55 [*]	0.58 [*]	0.65 [*]	0.64 [*]

^{*} Significantly different between groups (p 0.01);

[§] Significantly different between groups (p 0.05).