T₂ mapping provides multiple approaches for the characterization of 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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Skeletal muscles of children with Duchenne muscular dystrophy (DMD) show enhanced susceptibility to damage and progressive lipid infiltration, which contribute to an increase in the MR proton transverse relaxation time (T₂). Therefore, the examination of T₂ changes in individual muscles may be useful for the monitoring of disease progression in DMD. In this study, we used the mean T₂, percentage of elevated pixels and T₂ heterogeneity to assess changes in the composition of dystrophic muscles. In addition, we used fat saturation to distinguish T₂ changes caused by edema and inflammation from fat infiltration in muscles. Thirty subjects with DMD and 15 age-matched controls underwent T₂-weighted imaging of their lower leg using a 3-T MR system. T₂ maps were developed and four lower leg muscles were manually traced (soleus, medial gastrocnemius, peroneal and tibialis anterior). The mean T₂ of the traced regions of interest, width of the T₂ histograms and percentage of elevated pixels were calculated. We found that, even in young children with DMD, lower leg muscles showed elevated mean T₂, were more heterogeneous and had a greater percentage of elevated pixels than in controls. T₂ measures decreased with fat saturation, but were still higher (P < 0.05) in dystrophic muscles than in controls. Further, T₂ measures showed positive correlations with timed functional tests (r = 0.23–0.79). The elevated T₂ measures with and without fat saturation at all ages of DMD examined (5–15 years) compared with 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 used effectively to characterize muscle tissue in children with DMD, even in the early stages of the disease. Therefore, T₂ mapping may prove to be clinically useful in the monitoring of muscle changes caused by the disease process or by therapeutic interventions in DMD. Copyright © 2012 John Wiley & Sons, Ltd.

Keywords: Duchenne muscular dystrophy; skeletal muscle; MRI; proton transverse relaxation time; T₂ 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 noninvasive biomarkers that can provide global information on changes in composition of muscles caused by disease progression. Strength and functional tests are important clinical tools for the monitoring of the progression of disease in working muscle groups and in 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 children with DMD lose the ability to walk. Therefore, the adoption of additional outcome measures may be valuable for the study of the disease process and the effectiveness of therapeutic interventions in DMD.

MRI has become an important noninvasive tool for the study of muscle structure and composition over the last two decades, and has been successfully used to examine the disease status in children with DMD (4,6-17). One potential quantitative approach for the study of skeletal muscle in muscular dystrophies is the use of the proton transverse relaxation time ($T_2$) study of skeletal muscle in muscular dystrophies is the use of the $T_2$ range of ages. Furthermore, although $T_2$ may be more applicable for use as an outcome measure over a greater period, examination tends to be slower in the lower leg muscles and therefore may have an advantage over more proximal muscles, as disease progression is often more 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 implement fat suppression with $T_2$-weighted images. An elevated $T_2$ in fat-suppressed 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-suppressed (non-fatsat) and fat-suppressed (fatsat) $T_2$ MRI may help to elucidate the specific changes observed in $T_2$ of muscles in DMD.

Overall, the aims of this study were as follows: (i) to compare non-fatsat and fatsat $T_2$-based MRI measures (mean $T_2$, percentage of affected area and heterogeneity) in the lower leg muscles of children with DMD with those in controls; (ii) to evaluate the $T_2$-based MRI measures across different age groups in DMD and compare them with those in healthy controls; and (iii) to establish the relationships between $T_2$-based MRI measures and clinical functional tests.

**MATERIALS AND METHODS**

**Subjects**

In this cross-sectional study, 30 boys with DMD (5-15 years) 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 written consent.

**MR acquisition**

MR procedures were performed using a 3.0-T whole-body MRI scanner (Philips Achieva Quasar Dual 3 T, Philips, Amsterdam, the Netherlands) 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 2 days before MRI. Further, they were asked to use a wheelchair or similar mobility device when traveling (to the university or in airports) to avoid excessive walking. MR scanning of the right lower leg was performed using a SENSE eight-channel knee volume coil (field of view, 12–24 × 12–14 cm$^2$) 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

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**Table 1.** Comparison of healthy controls and subjects with Duchenne muscular dystrophy (DMD) with regard to various demographic features. Values are expressed as the mean ± standard deviation (SD) or as otherwise indicated.

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Controls ($n = 15$)</th>
<th>DMD ($n = 30$)</th>
<th>$p$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>9.7 ± 2.7</td>
<td>9.6 ± 2.5</td>
<td>0.873</td>
</tr>
<tr>
<td>Body weight (kg)</td>
<td>32.8 ± 10.4$^c$</td>
<td>32.02 ± 12.4</td>
<td>0.833</td>
</tr>
<tr>
<td>Height (cm)</td>
<td>139.7 ± 15.7$^c$</td>
<td>125.9 ± 13.1</td>
<td>0.010$^a$</td>
</tr>
<tr>
<td>Body mass index (kg/m$^2$)</td>
<td>18.2 ± 7.1$^c$</td>
<td>19.7 ± 4.1</td>
<td>0.016$^b$</td>
</tr>
<tr>
<td>Nonambulatory/ambulatory</td>
<td>0/15</td>
<td>2/30</td>
<td>NA</td>
</tr>
<tr>
<td>Brooke lower extremity score (median)</td>
<td>1</td>
<td>2 (1–9)</td>
<td>NA</td>
</tr>
</tbody>
</table>

NA, not applicable.

$^a$Significantly different between groups, $p \leq 0.01$.

$^b$Significantly different between groups, $p \leq 0.05$.

$^c$Information available for 14 subjects only.
placed around the proximal half of the lower leg (measured from the calcaneus to the tibial plateau) to capture the maximum cross-sectional area \( (CSA_{\text{max}}) \) of the triceps surae, peroneus and tibialis anterior (TA) muscle. \( T_2 \)-weighted spin echo (SE) images (12–18 axial slices; slice thickness, 7 mm; TR = 3 s; TE, five echoes evenly spaced between 20 and 100 ms) with saturation (spectral presaturation with inversion recovery) and without fat saturation were acquired with a refocusing angle of 120°. Fatsat data were collected for 10 healthy subjects; however, in two muscles [peroneal (PER) and medial gastrocnemius (MG)] of two healthy subjects, there appeared to be artifacts in the images, and therefore these 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 been used previously in ambulatory boys with DMD to assess lower extremity function (4,24–28). Of the 15 controls, we collected functional data on 14 boys, as one subject did not participate in functional testing because of time constraints. The timed functional tasks included walking 30 ft (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. Of the 30 subjects with DMD, four subjects did not complete the supine to stand activity. Among these four, three did not climb stairs and two could not participate in any of the functional tests, as they were nonambulatory. In addition to timed functional tests, each subject was also scored on the Brooke Lower Extremity Functional Scale (24).

**MRI data analysis**

\( T_2 \) mapping was performed using the axial slices of the lower leg in the region of \( CSA_{\text{max}} \) 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 \( CSA_{\text{max}} \) 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 monoexponential decay model to four TEs (40, 60, 80 and 100 ms) using a custom-written IDL software. As a result of 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), MG, TA, 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 TA and PER were significantly more heterogeneous than the other leg muscles in control as well as DMD groups. We speculated that some of this heterogeneity might be attributed to the 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, although the exclusion of fascia did not affect the mean \( T_2 \) of both muscles (PER: 50.8 versus 50.3 ms; TA: 45.5 versus 46.0 ms), it resulted in a reduced...
heterogeneity of the muscles (PER: 15.2 versus 10.0 ms; TA: 13.5 versus 8.6 ms). The heterogeneity of the muscles was quantified by measuring the widths of $T_2$ histograms (a further explanation of the technique is provided later in the text). As our main variable of interest was the muscle tissue itself, and not the fascia, the analyses without the fascia were used for the main comparisons in this study. The non-fatsat and fatsat images were processed separately but, as the images were acquired with a similar geometry (i.e. field of view and number of slices), the same regions of interest (ROIs) were used in non-fatsat and fatsat images.

For every muscle, $T_2$ values for each pixel within the traced ROI 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 involvement of children with DMD 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 histogram was used as threshold (Fig. 1). We determined the optimal threshold for the measurement of elevated $T_2$ values in dystrophic muscle by comparing the thresholds set at various levels between the 90th and 100th 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, and the lower values tended to result in 100% of the pixels being above the threshold in more involved muscles, and therefore were not 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 the heterogeneity of individual control muscles was greater than the heterogeneity of the mean $T_2$ values of the population. We also used a percentile rather than a standard deviation (SD) (e.g. 25SD), as 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, 48.0 ms; MG, 46.0 ms; PER, 49.0 ms; TA, 44.0 ms; fatsat: SOL, 46.0 ms; MG, 43.5 ms; PER, 44.0 ms; TA, 42.5 ms). Pixels with $T_2$ values above the threshold (95th percentile) in the muscles of interest were considered to be elevated and were expressed as a percentage (%) of the 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 the full width at half-maximum (FWHM) and full width at quarter-maximum (FWQM), as shown in Fig. 1.

### Statistical analysis

A nonparametric test (Mann–Whitney U-test) was performed to compare the mean $T_2$ percentage of elevated pixels above the threshold, FWHM and FWQM across the 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 (two-tailed) values ($p$) were reported for all the comparisons. For functional data analysis, the subjects with DMD who were unable to perform any functional tests 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 $p \leq 0.05$.

### RESULTS

#### $T_2$-based MRI measures

The non-fatsat mean $T_2$ of all four lower leg muscles was significantly higher ($p \leq 0.001$) in subjects with DMD in comparison with controls (Table 2; Fig. 1). As expected, mean $T_2$ values decreased with fat saturation, and the fatsat mean $T_2$ was elevated significantly ($p \leq 0.001$) in dystrophic muscles when compared with controls. On average, the non-fatsat $T_2$ was 10% higher than the fatsat $T_2$ in subjects with DMD ($p \leq 0.001$; Fig. 2).

The affected muscle area was quantified using a thresholding technique and was represented by the percentage of $T_2$ pixels elevated above the threshold value for the muscle in unaffected healthy subjects. The percentage of the affected area was significantly higher for all muscles in subjects with DMD compared with controls in both non-fatsat and fatsat data ($p \leq 0.001$; Fig. 2). A large variability was observed in the percentage of 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 subjects with DMD were found to be more heterogeneous in composition in comparison with controls (Table 3). For all muscles tested, the histograms of subjects with DMD were shifted right and demonstrated increased widths in comparison with controls (SOL = MG > PER > TA, $p \leq 0.01$). 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 subjects with DMD ($p \leq 0.001$).

#### Differences in $T_2$-based MRI measures across age groups

The children with DMD were divided into three groups: 5–7.9 years ($n = 12$), 8–11.9 years ($n = 14$) and 12–15 years ($n = 4$). For the analysis

<table>
<thead>
<tr>
<th>Muscle</th>
<th>Non-fatsat</th>
<th>Fatsat</th>
</tr>
</thead>
<tbody>
<tr>
<td>SOL</td>
<td>40.3 ± 1.1</td>
<td>50.6 ± 5.5a</td>
</tr>
<tr>
<td>MG</td>
<td>39.2 ± 1.9</td>
<td>50.8 ± 7.9a</td>
</tr>
<tr>
<td>PER</td>
<td>39.7 ± 1.5</td>
<td>51.2 ± 8.4a</td>
</tr>
<tr>
<td>TA</td>
<td>38.7 ± 1.3</td>
<td>45.7 ± 5.0a</td>
</tr>
</tbody>
</table>

*Significantly different between groups ($p \leq 0.001$)
of age-related differences in $T_2$-based MRI measures, subjects with DMD in each age group were compared with healthy controls. The mean $T_2$ and percentage of elevated pixels were significantly higher ($p \leq 0.01$) for muscles in DMD in all age groups (Fig. 3). Among measures of heterogeneity, FWQM was relatively more sensitive than FWHM for the elicitation of differences between dystrophic and control muscles, as well as within dystrophic muscles across different age groups. Overall, the dystrophic muscles were more heterogeneous than control muscles 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 $T_2$ and heterogeneity than the other lower leg muscles in younger age groups in both non-fatsat and fatsat images (5–8 and 8–12 years, $p \leq 0.05$).

**DISCUSSION**

In this study, we exploited $T_2$ 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 $T_2$ measures to evaluate the percentage of the affected region and the heterogeneity within and among muscles. Our main findings were as follows: (i) $T_2$ values were elevated in the lower leg muscles of children with DMD in comparison with controls in all age groups examined, including young subjects (5–8 years); (ii) $T_2$ was also elevated in DMD with fat saturation applied, indicating that factors other than an increase in lipid content contribute to elevated $T_2$; (iii) the use of $T_2$ histograms with a thresholding technique to estimate the percentage of affected muscle and the measurement of FWHM/FWQM as an index of muscle heterogeneity provide additional measures of monitoring disease involvement in children with DMD; and (iv) $T_2$ measures, including alternative $T_2$-based approaches (i.e. percentage of elevated pixels and heterogeneity), are strongly correlated with functional abilities in this population.

**Multiple muscles and age groups**

This study evaluated the involvement of multiple muscles of the lower leg in children with DMD using various $T_2$-based approaches.
The advancement of disease is typically slower in the lower leg muscles relative to those of 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 the monitoring of disease progression and treatment throughout the lifespan of children with DMD. Our results showed that $T_2$ was elevated in the SOL, MG, PER and TA muscles compared with age-matched controls. We found considerable variability in terms of muscle involvement among

![Figure 3. Comparison of mean $T_2$ in peroneal (PER) (a) and tibialis anterior (TA) (b) muscles between controls and children with Duchenne muscular dystrophy (DMD) in different age groups. Changes in percentage of elevated pixels across different age groups in PER (c) and TA (d) muscles in children with DMD in comparison with controls. Boxes represent the 25th and 75th percentiles and bars indicate the 5th and 95th percentiles with the median represented by a horizontal line within the DMD box plots. **Significantly different between groups, $p \leq 0.001$. *Significantly different between groups, $p \leq 0.01$. $^*$Significantly different between groups, $p \leq 0.05$.](image)

### Table 4. Comparison of measures of heterogeneity [full width at half-maximum (FWHM) and full width at quarter-maximum (FWQM)] between controls and subjects with Duchenne muscular dystrophy (DMD) in different age groups

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

Fatsat, fat-saturated; MG, medial gastrocnemius; Non-fatsat, non-fat-saturated; PER, peroneal; SOL, soleus; TA, tibialis anterior. Values are expressed as mean ± standard deviation (SD).

- $^a$Significantly different between groups, $p \leq 0.001$.
- $^b$Significantly different between groups, $p \leq 0.01$.
- $^c$Significantly different between groups, $p \leq 0.05$. 

Table 5. Comparison of T2-based MRI measures with functional tasks. Correlation coefficients (r) for various comparisons are reported. Subjects who were unable to perform any functional activity were given the highest score for rank correlations.

<table>
<thead>
<tr>
<th></th>
<th>Mean T2</th>
<th>Elevated pixels (%)</th>
<th>FWHM</th>
<th>FWQM</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>SOL</td>
<td>MG</td>
<td>PER</td>
<td>TA</td>
</tr>
<tr>
<td>30-ft walk</td>
<td>0.42b</td>
<td>0.57a</td>
<td>0.61a</td>
<td>0.59a</td>
</tr>
<tr>
<td>Supine-up</td>
<td>0.63b</td>
<td>0.69a</td>
<td>0.77a</td>
<td>0.72a</td>
</tr>
<tr>
<td>Stairs</td>
<td>0.52a</td>
<td>0.64a</td>
<td>0.72a</td>
<td>0.63a</td>
</tr>
<tr>
<td>Brooke score</td>
<td>0.62a</td>
<td>0.64a</td>
<td>0.75a</td>
<td>0.70a</td>
</tr>
</tbody>
</table>

FWHM, full width at half-maximum; FWQM, full width at quarter-maximum; MG, medial gastrocnemius; PER, peroneal; SOL, soleus; TA, tibialis anterior.

*aSignificantly different between groups, p ≤ 0.01.

**bSignificantly different between groups, p ≤ 0.05.

Figure 4. Example plots showing the correlation of the percentage of elevated T2 pixels in the tibialis anterior (TA) muscle with the time to walk 30 ft (a) and the time to stand from supine (b). Subjects not able to complete the task are not included in the figure.

In children with DMD within the same age groups, overall, there was a trend of a progressive increase in non-fat-sat T2-based MRI measures in the dystrophic muscles across age groups (Fig. 3). Importantly, even the young age group (aged 5–8 years) showed significantly elevated T2 measures in dystrophic muscles in comparison with controls. Therefore, T2 may have an advantage over other outcome measures typically used to investigate 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 T2 has been reported previously in the TA (18) and thigh (12) muscles of children with DMD. The present study extends these findings by showing differences among the lower leg muscles, including lower mean T2 values in the TA than SOL, MG and PER muscles, particularly in younger age groups, in DMD. Therefore, TA may be a 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 muscle strength before plantarflexors via manual muscle strength testing (30). However, our results are supported by recent imaging studies (computed tomography/MR), which also found the relative sparing of TA compared with other muscles of the lower leg (16,17,31). Furthermore, a previous study from our laboratory on the age-related differences in CSA and strength measures showed that the specific torque of the plantarflexor muscles was impaired fourfold in boys with DMD compared with healthy subjects, whereas the deficit in specific torque of dystrophic dorsiflexors was only twofold lower than in control muscles (14). Therefore, this study also supports the relative sparing of the TA muscle in DMD. Similarly, selective sparing of some muscles (gracilis, sartorius and semitendinosus) has also been observed in the upper leg (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 the 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 the 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 T2 was significantly higher in dystrophic muscle in comparison with 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 T2 in dystrophy (20). Furthermore, we found that the percentage decrease in these T2-based MR measures after fat saturation was much greater in muscles of older than younger children with DMD. In addition, fatsat T2 values tended to increase by a smaller amount with an increase in the age categories in comparison with non-fat-sat T2 values. These results confirm that, with disease progression, lipid infiltration increases in DMD.

Interestingly, after fat saturation, the TA muscle showed a trend towards a progressive decrease in T2-based MRI measures across increasing age groups in contrast with other muscles (Fig. 3). This may be a result of fewer edematous changes with age or an increase in fibrotic tissue in older children with DMD. Notably, PER had a comparatively higher threshold than other muscles in healthy controls in non-fat-suppressed images (50 ms), but the threshold for the PER muscle dropped by 6 ms (44 ms) in fat-suppressed images. These results indicate that the composition of even healthy PER muscles may be different from other muscles of the lower leg with the presence of more fat.

It should be noted that, although fat saturation effectively eliminates the majority of lipid signal from the images, it typically does not completely remove the fat contribution homogeneously. Therefore, one may argue for the possibility of a fat contribution to
the elevated \( T_2 \) values after fat saturation in DMD relative to controls. However, our results showed that, even in young subjects, where changes from fat infiltration are expected to be minimal, \( T_2 \) measures were still elevated in DMD relative to controls. Therefore, it appears that elevated \( T_2 \) in the fatsat data of subjects with DMD is not simply a result of incomplete fat saturation. Future studies aimed at the quantification of the contribution of lipid to the progression of disease in DMD using a three-point Dixon technique may be valuable.

**Alternative \( T_2 \)-based MRI approaches**

In this study, we showed that \( T_2 \) histograms provide an alternative approach to quantify muscle involvement in DMD. We used an objective thresholding technique for the quantification of the percentage of the affected area in dystrophic muscles. We found that, in comparison with the conventional method of comparing \( T_2 \)-maps, the mean \( T_2 \) of affected areas provided a greater range of values for monitoring the changes in dystrophic muscles (40–85 ms versus 0–100%; Fig. 3). Therefore, we anticipate that the latter method may be more sensitive than the mean \( T_2 \) measure for following the subsequent minor changes within the dystrophic muscles in response to the 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 the threshold was defined as the cross-sectional area of fat (FCSA) in the lower leg muscles of adult patients with dystrophy. However, that study did not consider that \( T_2 \) may also be elevated in dystrophic muscles as a result of inflammation, fluid accumulation and shifts in water compartmentalization (37,38). Moreover, our methodology for the calculation of the threshold \( T_2 \) value was different from that used in the previous study. In that study, the authors pooled the data from the TA, SOL and gastrocnemius muscles of three normal subjects to produce a group mean signal intensity, and used the 95th percentile of the normal muscle signal as a common threshold value for the 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 muscles separately. We found that PER had the highest threshold and TA had the lowest threshold among the chosen muscles of the lower leg. Therefore, if we had used the previous technique, we may have overestimated the percentage of elevated pixels in dystrophic PER muscles or underestimated the affected area in other muscles, especially TA.

We also evaluated the widths of the \( T_2 \) histograms to quantify muscle heterogeneity in children with DMD. Previous studies have shown DMD to be characterized by fibrosis, lipid and inflammation (11,38). As edema/inflammation and fat infiltration have longer \( T_2 \) values than healthy muscle tissue, and fibrosis lies on the opposite side of the \( T_2 \) spectrum (i.e. shorter mean \( T_2 \)), together these pathophysiological changes will result in a greater distribution of \( T_2 \) within dystrophic muscles. The heterogeneity in dystrophic muscles has been reported previously by Wansapura et al. (15). These authors found that the measurement of the heterogeneity with \( T_2 \) histograms was sensitive to early myocardial changes, even when the mean \( T_2 \) in subjects with DMD remained unchanged. However, in our study, both the mean \( T_2 \) and measures of heterogeneity were able to detect differences in the muscles of young children with DMD. Therefore, the quantification of tissue heterogeneity may provide additional information about the change in composition of muscle tissue in DMD. Importantly, the assessment of the \( T_2 \) distribution may be useful in the stages in which muscle is replaced by fibrotic tissue, thereby nullifying any overall increase in mean \( T_2 \) from edema or fat infiltration.

Future, 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 a broader distribution near the base of the histogram (Fig. 1e). Therefore, we hypothesized that FWQM may be more sensitive than \( T_2 \) to distribution changes. We found that FWQM rather than \( T_2 \) showed comparatively stronger correlations in MG, TA and PER with various functional tests, and this measure of heterogeneity was relatively more sensitive than \( T_2 \) 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 the mean \( T_2 \) and alternative \( T_2 \)-based MR approaches were strongly related to the functional tests, specifically the supine-up test and Brooke score. These findings were in accordance with previous studies that showed a positive correlation between the mean \( T_2 \) of dystrophic muscles and clinical assessments, i.e. the supine to stand and 30-ft walk tests in DMD (12). This further strengthens the validity of using \( T_2 \) measures as an additional clinical tool for monitoring the changes in the individual muscles in this population.

**CONCLUSION**

The results of this study show that \( T_2 \) mapping provides multiple approaches that can be used effectively to characterize muscle tissue in children with DMD employing a combination of fatsat and non-fatsat images. This approach enabled the measurement of muscle involvement using the mean \( T_2 \) of multiple muscles and the percentage of the affected muscle area and provided an index of muscle heterogeneity. Therefore, we anticipate that \( T_2 \) mapping could be used as a noninvasive and sensitive biomarker for the quantification of early and subtle muscle changes caused by the 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 to provide further information on the potential of these \( T_2 \) analysis strategies for the monitoring of disease progression in DMD.

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