Quantitative Muscle Ultrasound versus Magnetic Resonance Imaging in Duchenne Muscular Dystrophy

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Keywords: Ultrasonography, Magnetic resonance imaging, Duchenne muscular dystrophy, Child

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Abstract

Background: Nowadays, it needs favorable biomarkers to follow up the disease progression and therapeutic responses of Duchenne muscular dystrophy (DMD). This study evaluates which one of Quantitative muscle ultrasound (QMUS) and magnetic resonance imaging (MRI) is suitable for the disease in China.

Methods: Thirty-six boys with DMD engaged in the longitudinal observational cohort study, who used prednisone from baseline to 12th month. Muscle thickness (MT) and echo intensity (EI) of QMUS and T1-weighted MRI grading were measured in the right quadriceps femoris of the boys with DMD.

Results: The scores of MT and EI of QMUS and T1-weighted MRI grading showed significant correlations with the clinical ones of muscle strength, timed testing, and quality of life. The scores of MT and EI of QMUS showed good correlations with the ones of T1-weighted MRI grading too (P<0.05). But 15 of 36 boys with DMD did not take MRI examinations for different reasons.

Conclusions: QMUS and MRI can use as biomarkers for tracking DMD. Nevertheless, QMUS, because of its practical, low cost, and patient-friendly, applies for DMD widely than MRI in China.

Keywords: Ultrasonography, Magnetic resonance imaging, Duchenne muscular dystrophy, Child

Background

Duchenne muscular dystrophy (DMD; OMIM 310200) is an x-linked disease, with an incidence of 1:3,600 - 6,000 live male births. DMD is characterized by progressive muscle weakness, loss of walk independently, finally death from respiratory or cardiac failure [1]. Although they are useful to diagnose and track the disease progression of DMD, standard measures are still constrained by the patients’ effort and mood. They cannot perform in boys of all ages and abilities [2,3]. In contrast, muscle imaging is a reliable technique for quantifying muscle pathology of DMD, which can perform at all ages and does not rely on the patient’s effort.

QMUS using gray-scale analysis takes advantage of reference values for each muscle correcting for age and weight-related changes in echo intensity (EI) and the innate differences between individual muscles. Increased intramuscular fat and connective tissue result in higher EI of QMUS in the boys with DMD. QMUS has high predictive values of screening for DMD and is a promising longitudinal follow-up tool [4,5]. T1- weighted images of MRI can detect fatty infiltration, which signal is null. The signal hyperintensity suggests the presence of edema and/or inflammation. Fibrotic tissue displays no signal intensity and appears as a black spot. MRI is a powerful and sensitive technique to follow up the disease progression and therapeutic responses of DMD [6]. It is a good correlation between QMUS and MRI to measure atrophy and fatty infiltration of supraspinatus muscle [7].
In this study, we compare QMUS and MRI for depicting the longitudinal changes of the right quadriceps femoris (QF) muscle in 36 boys with DMD. And then, we evaluate which is suitable to follow up the disease progression and therapeutic responses of DMD in China.

**Methods**

*Patients*

The longitudinal observational cohort study was conducted at Children’s Hospital, Chongqing Medical University, China. Thirty-six boys with DMD were confirmed by dystrophin gene testing or muscle biopsy. They walked independently and engaged in the study between December 2010 and December 2012 [8]. The boys with DMD visited the clinic regularly for QMUS, MRI and clinical data collection. Before two days of the visit, the boys avoided any of their exceeded normal physical activities. Written informed consents were obtained from their parents or caregivers. The study was carried out in compliance with the Helsinki Declaration and approved by the Ethics Committee of Children’s Hospital, Chongqing Medical University.

*Clinical evaluations*

Clinical evaluations were at the beginning, 6th and 12th months of prednisone treatment which was 0.75 mg/kg/day for 12 months. Muscle strength of right hip flexion and knee extension were assessed by the Medical Research Council (MRC) scale, expanding to 10-point [9, 10]. Timed testing included the time to walk 10 meters, climb 4 standard steps, and stand from the supine position on the floor (Gowers time). The results were in seconds [9, 10]. Quality of life (QoL) was measured by the Chinese version of the PedsQL™ 3.0 Neuromuscular Module[11]. Items rated on a 5-point scale and transformed linearly into a 0–100 scale. The order for testing, the interval between tests, and protocols were standardized for each subject.

*QMUS measurements*

QMUS measurements were a real-time ultrasonic scanner (SIEMENS ACUSON × 300 PE, Healthcare Sector, Erlangen, Germany) with an 8-MHz probe according to the previous reports [4,5]. All parameters kept constant during the study, including gain (70 dB), compression (55), and the time to gain compensation. QMUS marker placed at the right halfway along the line from the anterior superior iliac spine to the superior aspect of the patella. Electronic calipers measured muscle thickness (MT) of the right QF muscle. The computer-assisted grey-scale analysis determined the mean EI, ranging from 0 ( = black) to 255 ( = white). The normal QMUS values were established in the lab. The test protocols were standardized for each subject.

*MRI measurements [6, 7,12]*
MRI examinations were a 1.5T MR system (Siemens, Erlangen, Germany). The boys with DMD lying on his supine position of a two-channel surface coil, a body matrix array coil, or transmit-receive quadrature extremity coil used for thigh imaging. Transverse T1-weighted MRIs were acquired around the center of the femur (field of view 175×175 mm, base resolution 380, repetition time 500 ms, echo time 8 ms, number of slices 10, slice thickness 5 mm, slice gap 1 mm, acquisition time 5 min). The anatomical location of the right QF muscle used in MRI measurement was equal to the one of the QMUS marker. The series of MR images were reviewed on the picture archiving and communication system (PACS) workstations. The degree of muscle dystrophy was evaluated according to a modified 5-point scale [13].

Statistical Analysis

All statistical analyses were in SPSS 17.0 software (SPSS Inc., Chicago, IL, USA). Continuous variables presented as mean and standard deviation (mean ± SD). Changes of the clinical characteristics and QMUS from baseline to 12th month compared using one-way ANOVA, and MRI using a two-tailed t-test. Correlations between QMUS, MRI and clinical testing calculated using Pearson correlation coefficients (r) for continuous data and Spearman correlation coefficients (rs) for ordinal data. All P-values were 2-sided, and P<0.05 was considered a significant difference.

Results

Clinical characteristics

Thirty-six boys with DMD joined in the study. Among these, 15 boys ranged from 4–6 years, 15 ranged from 7–9 years, and 6 ranged from 10–12 years. Of note, 4 boys with DMD were excluded from the analysis of timed testing for they could not complete the 4 steps climb and Gowers test. Fifteen patients could not complete the child self-assessment survey PedsQL™3.0 Neuromuscular Module because they were under 7 years old at the start of the study. Besides, 7 individuals did not take MRI examinations as they did not cooperate with the inspection or their parents refused to do the examination. At 6 months thereafter, 2 patients were lost to follow up. At 12 months thereafter, 1 patient was lost to follow up, 2 dropped out due to loss of ambulation, and 8 did not take MRI examinations (table 1).

Changes in clinical characteristics, QMUS and MRI from baseline to 12th month

From baseline to 12th month, muscle strength and timed testing (walk 10 meters, climb 4 steps, and Gowers test) remained stable (F 0.09±0.86, P 0.43±0.91) in the boys with DMD. QoL improved gradually (F 3.33, 4.00; P 0.04, 0.02, respectively). MT and EI of QMUS in the right QF muscle showed a significant improvement (F 3.67, 13.17; P 0.03, <0.01, respectively) (table 1, figure 1). At the same time, T1-weighted MRI grading of the right QF muscle remained stable too (t 0.79, P 0.44) (table 1, figure 2).

Relationships between QMUS, MRI and clinical characteristics
Correlations between MT and EI of QMUS in the right QF muscle and the clinical characteristics, including muscle strength (MT, $r(s) = 0.44, 0.38; P = 0.02, 0.04$; EI, $r(s) = -0.43, -0.39; P = 0.02, 0.03$, respectively), timed testing (MT, $r(s) = -0.43, -0.36; P = 0.03, 0.06$; EI, $r(s) = 0.41, 0.43; P = 0.02, 0.03$, respectively), and QoL (MT, $r(s) = 0.37, 0.42; P = 0.08, 0.02$; EI, $r(s) = -0.48, -0.42; P = 0.02, 0.02$, respectively) were statistically significant in the boys with DMD. Similarly, correlations between T1-weighted MRI grading of the right QF muscle and muscle strength ($r(s) = -0.44, -0.47; P = 0.03, 0.01$, respectively), timed testing ($r(s) = 0.40, 0.55; P = 0.03 < 0.01$, respectively), and QoL ($r(s) = -0.50, -0.45; P = 0.03, 0.01$, respectively) were statistically significant too (tables 2 & 3).

Two-tailed Spearman correlation analysis showed good correlations between MT and EI of QMUS and T1-weighted MRI grading (MT, $r = -0.52, P = 0.02$; EI, $r = 0.53, P = 0.01$, respectively) of the right QF muscle in the boys with DMD (tables 2 & 3).

Discussion

Our results showed that MT and EI of QMUS and T1-weighted MRI grading of the right QF muscle had significant relations with the clinical characteristics in the boys with DMD who used prednisone from baseline to 12th month ($P < 0.05$). Besides, we found good correlations between MT and EI of QMUS and T1-weighted MRI grading in the right QF muscle ($P < 0.05$) (tables 1, 2 & 3). All the results proved that both QMUS and MRI can use as biomarkers to follow up the disease progression and therapeutic responses of DMD.

QMUS is a practical, patient-friendly, feasible, and quick tool (e.g., within 30 min for most procedures). It is not influenced by the patients’ condition, fatigability, and cooperation. In our study, QMUS was sensitive to the progressive changes of the muscle architecture of the boys with DMD between ages 4–12 years, even when they used prednisone (Figure 1). The changes in QMUS showed good correlations with the clinical characteristics. At the same time, all the boys with DMD were measured voluntarily by QMUS every 6 months during the clinical trial.

T1-weighted MRI gradings showed better than before with the improvements of the clinical characteristics in the boys with DMD who used prednisone. The changes of T1-weighted MRI gradings demonstrated the good correlations with the clinical characteristics in our study. Nevertheless, 4 boys with DMD showed asymmetric T1-weighted MRI grading (grade 2 changes) for their poor clinical characteristics at baseline. T1-weighted MRI gradings were still the same as before with the improvements of the clinical characteristics in the patients after using prednisone (Figure 2). The results were comparable to the previous study [14], in which the asymmetric individual muscle involvements were in the lower limb muscles of the boys with DMD.

A few limits and points still need to improve in our study. At first, QMUS is only suited for displaying superficial muscle layers, the reference values cannot set up reliably for deeper muscles. The second, QMUS cannot distinguish the abnormal signal intensities because of inflammation, fibrosis, or fatty
Infiltration [15]. Composite biomarkers, QMUS and impedance electromyography, may provide a more effective means for tracking the disease progression and therapeutic responses of DMD [16].

In our study, 15 of the 36 boys with DMD did not take MRI examinations for they did not cooperate with the inspection or their parents refused to do the examination because of economic hardship. This suggested the potential disadvantages of MRI including limited availability, claustrophobia, costs, and the need for sedation or anesthesia in young children. Such changes might affect the research results, which were the limits of MRI in developing countries.

In this study, the anatomical location of the right QF muscle performed in MRI measurement was equal to the QMUS marker. It was a limited protocol that QMUS and MRI only measured the right QF muscle for the sake of studying the two correlations easily. We did not detect the fatty infiltrations found by T1-weighted MRI at the same marker to the ultrasound of the QMUS “slice” in the boys with DMD. It means that, for disorders with uneven distribution of pathology along the muscle, the sensitivity of a single QMUS is limited compared to MRI. Thus, QMUS needs more slices (i.e., at more locations) to measure a muscle. The new reference values need to establish too for each specific location.

The limits of this study included the small sample size too, which 36 boys with DMD joined in the trial. Besides, the follow-up was not continued after 12 months. Most of the boys with DMD came to a large multicenter observational trial [8]. Therefore, further researches on learning how to deal with these limits should be carried out.

**Conclusions**

We further proved that both QMUS and MRI can use as biomarkers to follow up the disease progression and therapeutic responses of DMD. Nevertheless, QMUS, because of its practical, low cost, and patient-friendly, applies for DMD widely than MRI in China.

**List Of Abbreviations**
Declarations

Acknowledgments

We thank sincerely the children and parents who took part in this study. We are also grateful to all the experts involved in the study and support from the Children's Hospital, Chongqing Medical University.

Authors’ contributions

JH conceptualized and designed the study, statistical analyses, wrote the paper; LJ supervised the data analysis, and revised the manuscript; SH and CL collected human sera and performed clinical assessments; QW performed QMUS and statistical analyses; PX performed MRI and statistical analyses; JQ and LZ performed dystrophin gene testing or muscular biopsy. All authors read and approved the final manuscript.

Funding

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Ethics approval and consent to participate

Written informed consents were obtained from their parents or caregivers. The study was carried out in compliance with the Helsinki Declaration and approved by the Ethics Committee of Children's Hospital,
Chongqing Medical University.

**Consent for publication**

Written informed consents for publication were obtained from their parents or caregivers.

**Availability of data and materials**

All data generated or analyzed during this study are included in this published article and are available from the corresponding author on reasonable request.

**Competing interests**

The authors declare that they have no competing interests.

**References**


Tables

Table 1 Changes in clinical characteristics, QMUS and MRI from baseline to 12th month (n, mean ± SD)
<table>
<thead>
<tr>
<th>Clinical characteristics</th>
<th>n</th>
<th>baseline</th>
<th>n</th>
<th>6th month</th>
<th>n</th>
<th>12th month</th>
<th>F(1)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lower limb muscle strength (grade)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hip flexion: R</td>
<td>36</td>
<td>6.94 ± 0.89</td>
<td>34</td>
<td>6.88 ± 0.95</td>
<td>31</td>
<td>6.84 ± 0.99</td>
<td>0.10</td>
<td>0.91</td>
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<tr>
<td>Knee extension: R</td>
<td>36</td>
<td>7.20 ± 1.01</td>
<td>34</td>
<td>7.26 ± 0.86</td>
<td>31</td>
<td>7.16 ± 0.96</td>
<td>0.09</td>
<td>0.91</td>
</tr>
<tr>
<td>Timed functional testing (s)</td>
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<td></td>
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<td></td>
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<tr>
<td>Time to walk 10 meters</td>
<td>36</td>
<td>10.16 ± 1.82</td>
<td>34</td>
<td>9.78 ± 1.83</td>
<td>31</td>
<td>9.55 ± 2.16</td>
<td>0.86</td>
<td>0.43</td>
</tr>
<tr>
<td>Time to climb 4 steps</td>
<td>32</td>
<td>7.50 ± 2.80</td>
<td>30</td>
<td>6.70 ± 3.06</td>
<td>27</td>
<td>7.15 ± 3.12</td>
<td>0.56</td>
<td>0.58</td>
</tr>
<tr>
<td>Time for Gowers test</td>
<td>32</td>
<td>8.01 ± 2.78</td>
<td>30</td>
<td>7.39 ± 3.22</td>
<td>27</td>
<td>7.42 ± 3.19</td>
<td>0.40</td>
<td>0.67</td>
</tr>
<tr>
<td>PedsQL™ 3.0 NMM (total score)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Child self-report</td>
<td>21</td>
<td>56.74 ± 11.42</td>
<td>23</td>
<td>64.92 ± 14.58</td>
<td>24</td>
<td>67.38 ± 16.20*</td>
<td>3.33</td>
<td>0.04</td>
</tr>
<tr>
<td>Parent proxy-report</td>
<td>36</td>
<td>55.76 ± 13.01</td>
<td>34</td>
<td>63.46 ± 15.10</td>
<td>31</td>
<td>65.33 ± 16.53*</td>
<td>4.00</td>
<td>0.02</td>
</tr>
<tr>
<td>QMUS of QF muscle</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Muscle thickness (mm)</td>
<td>36</td>
<td>19.38 ± 4.21</td>
<td>34</td>
<td>21.47 ± 3.98</td>
<td>31</td>
<td>22.03 ± 4.63*</td>
<td>3.67</td>
<td>0.03</td>
</tr>
<tr>
<td>Echo intensity</td>
<td>36</td>
<td>77.36 ± 8.59</td>
<td>34</td>
<td>70.24 ± 10.13**</td>
<td>31</td>
<td>65.71 ± 9.47**</td>
<td>13.17</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>MRI of QF muscle (grade)</td>
<td>29</td>
<td>3.07 ± 1.00</td>
<td>21</td>
<td>2.86 ± 0.85</td>
<td></td>
<td></td>
<td>0.79</td>
<td>0.44</td>
</tr>
</tbody>
</table>

Note: Muscle strength—lower limb muscle groups (Right Hip flexion and Knee extension) were graded according to the MRC grading system. Timed functional testing – the time to walk 10 meters, to climb 4 standard steps, and to stand from supine position on the floor (Gowers time). Quality of life (QoL) questionnaire— the Chinese version of the PedsQL™ 3.0 Neuromuscular Module. Scale scores are computed as sum of the items divided by the number of items answered. QMUS of QF muscle—Quantitative muscle ultrasound of quadriceps femoris, including muscle thickness and echo intensity. MRI of QF muscle—Magnetic resonance imaging of quadriceps femoris, evaluated according to a modified 5-point scale.
Data are presented as mean ± SD and number. One-way ANOVA and two-tailed t test were used to compare the variability. *P<0.05, **P<0.01, compared to before treatment. a two-tailed t test.

SD, standard deviation; n, number; R, right; s, second; mm, millimeter

Table 2 Correlations between the baseline of clinical characteristics, QMUS and MRI

<table>
<thead>
<tr>
<th>Clinical characteristics</th>
<th>QMUS of QF muscle</th>
<th>MRI of QF muscle (grade)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Muscle thickness (mm)</td>
<td>r (rs)</td>
</tr>
<tr>
<td></td>
<td>Echointensity</td>
<td>r (rs)</td>
</tr>
<tr>
<td>Lower limb muscle strength (grade)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hip flexion: R</td>
<td>0.39a 0.02</td>
<td>-0.35a 0.04</td>
</tr>
<tr>
<td>Knee extension: R</td>
<td>0.28a 0.14</td>
<td>-0.40a 0.02</td>
</tr>
<tr>
<td>Timed functional testing (s)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Time to walk 10meters</td>
<td>-0.37 0.03</td>
<td>0.38 0.02</td>
</tr>
<tr>
<td>Time to climb 4 steps</td>
<td>-0.30 0.10</td>
<td>0.38 0.03</td>
</tr>
<tr>
<td>Time for Gowerstest</td>
<td>-0.46 0.02</td>
<td>0.53 &lt;0.01</td>
</tr>
<tr>
<td>PedsQL™ 3.0 NMM (total score)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Child self-report</td>
<td>0.51 0.02</td>
<td>-0.49 0.02</td>
</tr>
<tr>
<td>Parent proxy-report</td>
<td>0.39 0.02</td>
<td>-0.36 0.03</td>
</tr>
<tr>
<td>MRI of QF muscle (grade)</td>
<td>-0.44a 0.02</td>
<td>0.48a &lt;0.01</td>
</tr>
</tbody>
</table>

Note: Muscle strength—lower limb muscle groups (Right Hip flexion and Knee extension) were graded according to the MRC grading system. Timed functional testing – the time to walk 10meters, to climb 4 standard steps, and to stand from supine position on the floor (Gowers time). Quality of life (QoL) questionnaire– the Chinese version of the PedsQL™ 3.0 Neuromuscular Module. Scale scores are computed as sum of the items divided by the number of items answered. QMUS of QF muscle—Quantitative muscle ultrasound of quadriceps femoris, including muscle thickness and echo intensity. MRI of QF muscle—Magnetic resonance imaging of quadriceps femoris, which was evaluated according to a modified 5-point scale.
Correlations between QMUS, MRI and clinical characteristics were calculated using Pearson correlation coefficients ($r$) for continuous data and Spearman correlation coefficients ($r_s$) for ordinal data. \textsuperscript{a}Spearman correlation test.

R, right; s, second; mm, millimeter

Table 3 Correlations between the changes of clinical characteristics, QMUS and MRI at 12\textsuperscript{th} month

<table>
<thead>
<tr>
<th>Clinical characteristics</th>
<th>QMUS of QF muscle</th>
<th>MRI of QF muscle (grade)</th>
</tr>
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<tbody>
<tr>
<td></td>
<td>Muscle thickness (mm)</td>
<td>$r$ ($r_s$) $p$</td>
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<td></td>
<td>Echointensity</td>
<td></td>
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<tr>
<td></td>
<td>$r$ ($r_s$) $p$</td>
<td>$r$ ($r_s$) $p$</td>
</tr>
<tr>
<td>Lower limb muscle strength (grade)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hip flexion: R</td>
<td>0.44\textsuperscript{a} 0.02</td>
<td>-0.43\textsuperscript{a} 0.02</td>
</tr>
<tr>
<td>Knee extension: R</td>
<td>0.38\textsuperscript{a} 0.04</td>
<td>-0.39\textsuperscript{a} 0.03</td>
</tr>
<tr>
<td>Timed functional testing (s)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Time to walk 10meters</td>
<td>-0.39 0.04</td>
<td>0.42 0.02</td>
</tr>
<tr>
<td>Time to climb 4 steps</td>
<td>-0.43 0.03</td>
<td>0.43 0.03</td>
</tr>
<tr>
<td>Time for Gowerstest</td>
<td>-0.36 0.06</td>
<td>0.41 0.03</td>
</tr>
<tr>
<td>PedsQL\textsuperscript{TM} 3.0 NMM (total score)</td>
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</tr>
<tr>
<td>Child self-report</td>
<td>0.37 0.08</td>
<td>-0.48 0.02</td>
</tr>
<tr>
<td>Parent proxy-report</td>
<td>0.42 0.02</td>
<td>-0.42 0.02</td>
</tr>
<tr>
<td>MRI of QF muscle (grade)</td>
<td>-0.52\textsuperscript{a} 0.02</td>
<td>0.53\textsuperscript{a} 0.01</td>
</tr>
</tbody>
</table>

Note: Muscle strength – lower limb muscle groups (Right Hip flexion and Knee extension) were graded according to the MRC grading system. Timed functional testing – the time to walk 10meters, to climb 4 standard steps, and to stand from supine position on the floor (Gowers time). Quality of life (QoL) questionnaire – the Chinese version of the PedsQL\textsuperscript{TM} 3.0 Neuromuscular Module. Scale scores are computed as sum of the items divided by the number of items answered. QMUS of QF muscle – Quantitative muscle ultrasound of quadriceps femoris, including muscle thickness and echo intensity.
MRI of QF muscle—Magnetic resonance imaging of quadriceps femoris, evaluated according to a modified 5-point scale.

Correlations between QMUS, MRI and clinical characteristics were calculated using Pearson correlation coefficients ($r$) for continuous data and Spearman correlation coefficients ($r_s$) for ordinal data. *Spearman correlation test.

R, right; s, second; mm, millimeter

**Figures**

![Figure A](image1)

![Figure B](image2)

**Figure 1**

QMUS was sensitive to the progressive changes of the muscle architecture of the boys with DMD between ages 4-12 years, even when they used prednisone.
Figure 2

T1-weighted MRI gradings were still the same as before with the improvements of the clinical characteristics in the patients after using prednisone.