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T2 Mapping and Fat Quantification of Thigh Muscles in Children with Duchenne Muscular Dystrophy

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Summary: Quantitative magnetic resonance image (MRI) in individual muscles may be useful for monitoring disease progression in Duchenne muscular dystrophy (DMD). The purpose of this study was to measure T2 relaxation time of thigh muscles in children with DMD and healthy boys, and to correlate the T2 relaxation time of muscles with the fat fraction (FF) at quantitative magnetic resonance and results of clinical assessment. Thirty-two boys with DMD and 18 healthy boys were evaluated with T2 mapping and three-point Dixon MRI. Age, body mass index (BMI), muscle strength assessment, timed functional tests (time to walk or run 10 metres, rise from the floor and ascend four stairs), and the North Star Ambulatory Assessment (NSAA) were evaluated. Spearman's correlation was used to assess the relationships between FF and clinical assessments and T2 relaxation time. The mean T2 relaxation time of thigh muscles in DMD was significantly longer than that in the control group (P<0.05), except for the gracilis (P=0.952). The gracilis, sartorius and adductor longus were relatively spared by fatty infiltration in DMD patients. The T2 relaxation time was correlated significantly with the mean FF in all muscles. Age, BMI, total muscle strength score, timed functional tests and NSAA were significantly correlated with the overall mean T2 relaxation time. T2 mapping may prove clinically useful in monitoring muscle changes as a result of the disease process and in predicting the outcome of DMD patients.
Key words: T2 mapping; Duchenne muscular dystrophy; skeletal muscle; fat infiltration

Duchenne muscular dystrophy (DMD) is an X-linked recessive disorder that affects 1 in 3600–6000 live male births[1]. The disease is caused by loss-of-function mutations in gene coding for dystrophin, a protein located beneath the muscle fibre plasma membrane[2]. This abnormality results in inflammation, muscle fibre necrosis and replacement of fibres by connective and fatty tissue[3]. Boys with DMD suffer progressive muscle weakness that starts in the pelvic girdle; this spreads to the distal extremities. As the disease progresses, they often demonstrate a waddling gait and toe walking; the disease eventually results in the loss of the ability to rise from the floor, climb stairs, and ambulate independently by the age of 13 years. Death often occurs around the age of 19 as a result of respiratory and cardiac complications[1]. Corticosteroids are the only current pharmaceutical agent found to be beneficial in slowing disease progression in boys with DMD; these drugs are recommended as the standard of care once boys enter the plateau or decline disease phases[4].

In clinical practice, outcome measures in this area of research have been largely limited to muscle biopsies, measures of muscle strength and timed functional tests (time to walk or run 10 metres, rise from the floor and ascend four stairs)[5, 6]. However, muscle biopsy is invasive and it is limited to the site of sampling, and cannot be extended to all the muscles involved. Muscle strength and timed functional tests are important clinical tools for monitoring the disease progression in working muscle groups and understanding the disease pathology[7]. In addition to the muscle strength and timed functional tests, comprehensive motor evaluations have been proposed using a battery of functional tests, such as the North Star Ambulatory Assessment

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(NSAA), which uses a composite score of 17 items[8]. This score was recently shown to be sensitive for detecting differences between corticosteroid regimens in DMD[9]. However, these tests largely depend on patient collaboration, and comparability is impaired by fluctuations in the patient's condition. Therefore, there is a need for non-invasive biomarkers that can provide global information of changes in muscle composition resulting from disease progression.

Quantitative magnetic resonance image (qMRI) has become an accurate tool for the non-invasive detection of muscle structure and composition and has been successfully used to examine disease status and response to treatments in children with DMD[10–13]. Iterative decomposition of water and fat with echo asymmetry and least-squares estimation quantitation sequence (IDEAL-IQ) is a new method that can quantitatively evaluate fat infiltration by using three asymmetric echo time and the three-point Dixon method[14]. Recent researches show that IDEAL-IQ is used to estimate the fat fraction (FF) of liver, pancreas, bone marrow in human and vertebra in diabetic rabbits[15–17]. T2 relaxation time mapping (T2 mapping) is an MR imaging measurement of the time constant of decay of the nuclear MR signal[18]. Physiologic or pathologic macromolecular environmental changes of skeletal muscle can affect T2 relaxation time by alterations of water binding to neighboring molecules. The main disease processes of the skeletal muscles, namely inflammation/oedema and fatty infiltration, both increase T2 relaxation time. To our knowledge, there were few studies that combine T2 mapping and IDEAL-IQ to evaluate the thigh muscles in boys with DMD.

The purpose of this study was to quantitatively measure the T2 relaxation time and FF of thigh muscles in children with DMD and to correlate the overall T2 relaxation time of muscles with the FF and clinical assessments results.

1 MATERIALS AND METHODS

1.1 Participants

This prospective study was approved by the local institutional review board, and written informed consent was obtained from all subjects and their parents. Between August 2017 and May 2018, a cohort of 32 DMD boys (mean age, 8.4±1.9 years; range, 5.0–13.3 years) were included in this study. Among the DMD patients, 28 were ambulant and 4 were wheelchair-bound. Inclusion criteria were genetically and/or muscle biopsy-proven DMD diagnosis and no severe or moderate learning difficulties or behavioural problems. Exclusion criteria were a history of any other known medical disorders, including any other neuromuscular, metabolic or endocrine disorders that could alter bone or muscle metabolism; contraindications to MRI; and an inability to cooperate and participate in the various tests.

Eighteen healthy control boys (mean age, 9.3±1.9 years; range, 6.0–12.1 years) were recruited from local schools in the same period. Inclusion criteria for the control group were as follows: no history of neuromuscular disorder or muscle weakness, no family history of neuromuscular disorder, no systemic diseases potentially associated with fatty infiltration or inflammation of muscle, and functionally normal physical activity.

1.2 Clinical Assessments

All enrolled children underwent clinical assessments by a single investigator who was blinded to the MR imaging findings. The clinical assessments included the patient's age, height, weight, muscle strength, timed function tests and NSAA. All clinical assessments were performed within 1 week of the MRI examination. Body mass index (BMI) was calculated for each subject from measured height and weight values as weight in kilograms divided by the square of height in metres.

Muscle strength assessment was evaluated using a scale modification of the Medical Research Council Scale[19]. The power of major movements around the hips (flexion, adduction, abduction), knees (extension, flexion), and ankles (dorsiflexion, plantar flexion) was tested. A score of 5 was normal, and 0 meant the absence of any perceptible movement. For analysis purposes, this scale was converted to a 0–10 scale as follows: 0=0, l=1, 2=2, 3–=3, 3=4, 3+=5, 4, 4– and 4+=7, 5–=9, 5=10[20]. The term 'total muscle score' indicates the total score of all tested muscle groups.

Three functional tasks that were timed using a stopwatch included the time to walk or run 10 metres (10 m walk/run), rise from the floor (supine up), and ascend four stairs (4 stairs)[21]. Subjects were asked to perform each task three times as fast as they could; the average time was recorded for analysis. For those who were unable to perform the tasks, the functional time was set to an arbitrary maximum allowable time score (99 s)[22].

The NSAA is a functional scale specifically designed for ambulant boys affected by DMD[23]. The scale consists of 17 items, ranging from standing (item 1) to running (item 17). Each item can be scored on a 3-point scale using simple criteria: 2=ability to perform the test normally; 1=modified method or assistance to perform test; and 0=unable to perform the test. The total score can be achieved by summing the scores for all the individual items[24].

1.3 MR Acquisition

MRI examinations were performed with a 3.0 T MRI unit (GE Discovery 750, GE Healthcare, USA) with a 32-channel phased-array torso coil. The patients

underwent imaging in a supine position.

For IDEAL-IQ, the following pulse sequences were used: 6 echoes per repetition time, TE, 3.2 ms; TR, 6.9 ms; echo train length, 6; slice thickness, 5 mm; field of view, 30 cm×30 cm; matrix, 160×160; flip angle, 3°; number of excitation, 2; bandwidth, 111.11 kHz. Fifty sections were acquired by using these parameters from the iliac crest to femoral condyles. The acquisition time was approximately 1 min and 30 s.

Axial T2 mapping was performed with a multislice multiecho sequence without fat suppression. The following parameters were used: number of slices, 5; number of echoes, 8; TE/TR, 6.2, 12.4, 18.6, 24.9, 31.1, 37.3, 43.5, 49.7/500 ms; matrix, 320 × 256; number of excitation, 1; section thickness, 4 mm; section gap, 10–50 mm. The acquisition time for the T2 map sequences was approximately 2 min.

1.4 MR Analysis

The MR data were transferred to and analysed at an imaging workstation (Advantage Windows 4.6; GE Medical System, USA). Regions of interest (ROI) were drawn manually on FF maps and T2 maps according to an anatomical atlas for the 11 muscles in the left side of thigh: rectus femoris (RF), vastus intermedius (VI), vastus lateralis (VL), vastus medialis (VM), sartorius (Sar), adductor longus (AL), gracilis (Gra), adductor magnus (AM), semimembranosus (SM), semitendinosus (ST) and biceps femoris long head (BFLH). ROIs delineated the interior of the muscle avoiding fasciae and blood vessels. For ROI placement, two section levels were chosen because they contained the largest area of visible muscles with good differentiation of the different muscle compartments. The operators were blinded to the clinical information, the original MRI imaging report, and clinical examination during the evaluation. For each patient, the levels selected for evaluation were the following: for VF, VI, VL, Sar, AL, Gra, AM and ST, a section through the proximal 1/3 of the thigh; for VM, SM, BFLH, a section through the distal 1/3 of the thigh (fig. 1).

1.5 Statistical Analysis

Statistical analysis was performed with the commercially available software package SPSS 20.0 (IBM, USA). The normality of each variable was assessed using the Shapiro-Wilk test. Demographic information and muscle T2 relaxation time were expressed as the mean±standard deviation (SD). FF values were indicated with medians (interquartile range). Demographic information, muscle T2 relaxation time and FF values were compared between the DMD and control groups using unpaired *t*-tests/Mann-Whitney *U* tests. The Spearman correlation coefficient model was used to evaluate the correlation between mean T2 relaxation time, FF and clinical assessments (age, BMI, NSAA, total muscle strength score, timed functional tests). The upper limit for a 95% reference interval obtained from the healthy control group was applied to boys with DMD to establish the accuracy of T2 maps in the diagnosis of DMD. Positive and negative correlations were evaluated and considered significant if the *P* value was less than 0.05.

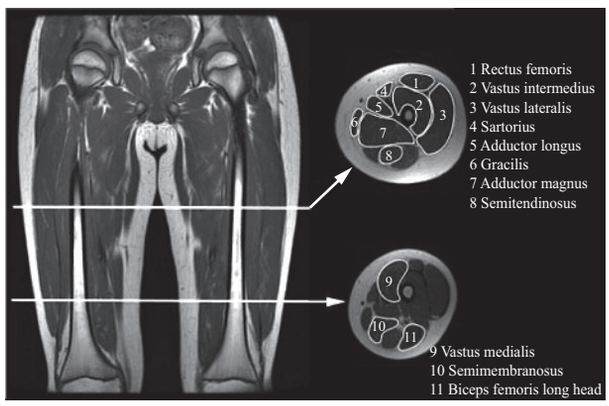

Fig. 1 Axial sections of the left thigh for a healthy volunteer, showing the muscles used and conservative margins for regions of interest

2 RESULTS

The descriptive characteristics of boys with DMD and the control subjects are presented in table 1. No significant difference was found between the DMD and control groups in terms of age (P=0.125). The control subjects were taller and had greater weight and higher BMI than those in the DMD group (P<0.05).

FF maps and T2 maps of control and DMD subjects are shown in fig. 2. The overall mean FF values were 10.9 (5.2, 31.2)% and 2.9 (1.8, 4.4)% in DMD and control boys, respectively. The mean FF of thigh muscles in DMD group was significantly higher than that in the control group (P<0.05), except for the

Table 1 Subject demographics of the DMD and control boys

Characteristics	DMD (n=32)	Controls (n=18)	P value
Age (years)	8.4±1.9	9.3±1.9	0.125
Height (m)	1.24±0.10	1.36±0.12	0.003*
Weight (kg)	27.0±9.4	34.3±7.2	0.001*
BMI (kg/m^2)	17.1±3.9	18.5±2.0	0.019*
Non-ambulatory/Ambulatory	4/32	0/18	NA

BMI: body mass index; NA: not applicable. *P<0.05

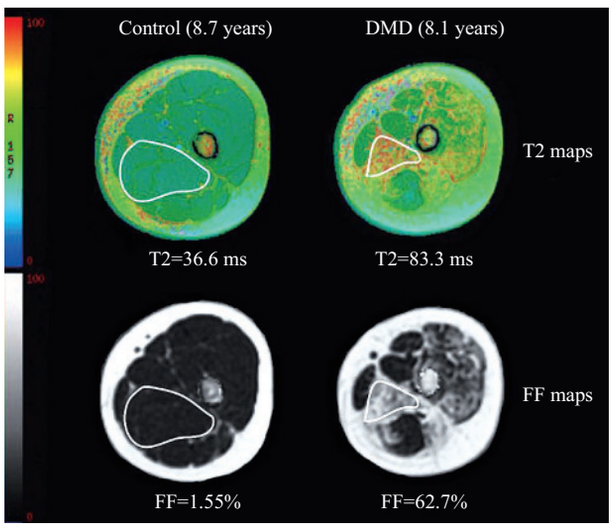

Fig. 2 T2 maps and FF maps of control (8.7 years) and DMD (8.1 years) subjects
The adductor magnus showed advanced fatty infiltration and atrophy in DMD boy. The T2 relaxation time of the adductor magnus in control and DMD groups was 36.6 ms and 83.3 ms, respectively. The FF in the adductor magnus in control and DMD groups was 1.55% and 62.7%, respectively.

Sar (P=0.069), AL (P=0.120), and Gra (P=0.332). The AM had the greatest mean FF values [34.8 (14.1, 59.8)%], followed by the quadriceps femoris (QF) and BFLH [13.1 (7.0, 45.6)% to 23.2 (9.0, 52.5)%]. The lowest mean FF values were documented in the Sar [6.9 (4.6, 12.5)%], AL [2.6 (1.5, 15.5)%], and Gra [5.1 (2.6, 8.0)%].

The overall mean T2 relaxation time was 60.7±16.1 ms and 41.1±4.4 ms in DMD and control boys, respectively. The mean T2 relaxation time of thigh muscles in DMD group was significantly longer than that in the control group except for the Gra (P=0.952). The AM had the longest mean T2 relaxation time (75.8±18.6 ms), followed by the QF and BFLH (58.3±13.8 ms to 69.5±15.1 ms). The shortest mean T2 relaxation time was documented in the AL (52.1±17.4 ms) and Gar (46.6±8.4 ms) (fig. 3, table 2).

To establish a normal range of T2 relaxation time for the thigh muscles, the upper limit for a 95% reference interval for muscles was obtained from the healthy boys. A cutoff value of 46.4 ms for the AM T2 relaxation time yielded complete separation between boys with DMD and healthy boys with 100% sensitivity and 88.9% specificity. A cutoff value of 46.5 ms for

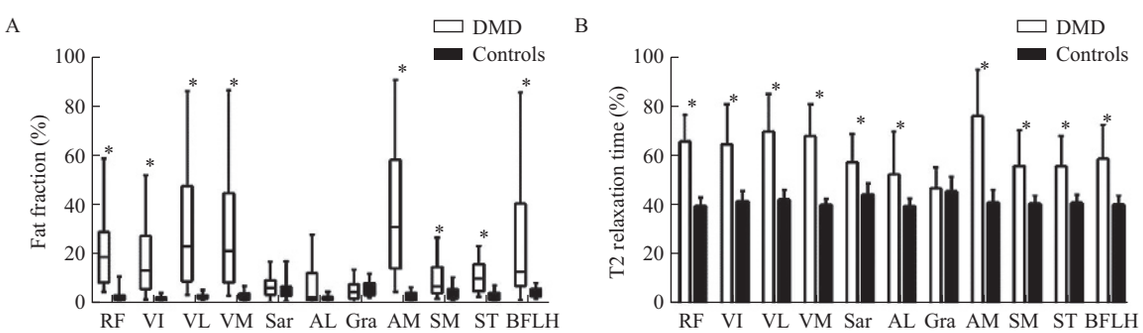

Fig. 3 Comparison of the mean FF (A) and T2 relaxation time (B) in thigh muscles between the control and DMD subjects
Boxes represent 25 and 75 percentiles and arrow bars indicate the 5 and 95 percentile with the median presented with a horizontal line within the DMD boxplots. T2 relaxation time is represented as mean±SD. RF: rectus femoris; VI: vastus intermedius; VL: vastus lateralis; VM: vastus medialis; Sar: sartorius; AL: adductor longus; Gra: gracilis; AM: adductor magnus; SM: semimembranosus; ST: semitendinosus; BFLH: biceps femoris long head. *P<0.05 *vs.* controls

Table 2 FF and T2 relaxation time in thigh muscles of the DMD and control boys

Muscle	Median FF (interquartile range)			T2 relaxation time (mean±SD)		
	DMD	Control	*P* value	DMD	Control	*P* value
RF	18.8 (7.9, 30.1)	2.5 (1.4, 3.4)	<0.001*	65.4±10.8	39.3±3.7	<0.001*
VI	13.5 (5.8, 39.7)	1.7 (1.3, 2.5)	<0.001*	64.4±16.2	41.1±4.2	<0.001*
VL	23.2 (9.0, 52.5)	2.5 (2.1, 3.3)	<0.001*	69.5±15.1	41.9±4.2	<0.001*
VM	21.3 (8.5, 46.3)	2.8 (1.7, 4.1)	<0.001*	67.6±13.0	39.8±2.5	<0.001*
Sar	6.9 (4.6, 12.5)	4.6 (3.1, 6.8)	0.069	57.0±11.6	44.0±4.6	<0.001*
AL	2.6 (1.5, 15.5)	1.8 (1.6, 2.6)	0.120	52.1±17.4	39.2±3.3	<0.001*
Gra	5.1 (2.6, 8.0)	6.8 (3.4, 8.2)	0.332	46.6±8.4	45.1±6.1	0.952
AM	34.8 (14.1, 59.8)	2.7 (1.5, 4.4)	<0.001*	75.8±18.6	40.7±5.2	<0.001*
SM	7.4 (4.2, 20.4)	3.3 (2.0, 5.8)	<0.001*	55.6±14.4	40.3±3.3	<0.001*
ST	10.0 (5.0, 20.9)	3.2 (1.5, 4.4)	<0.001*	55.5±12.0	40.5±3.6	<0.001*
BFLH	13.1 (7.0,45.6)	3.8 (2.9, 6.0)	<0.001*	58.3±13.8	39.9±3.9	<0.001*

RF: rectus femoris; VI: vastus intermedius; VL: vastus lateralis; VM: vastus medialis; Sar: sartorius; AL: adductor longus; Gra: gracilis; AM: adductor magnus; SM: semimembranosus; ST: semitendinosus; BFLH: biceps femoris long head. *P<0.05

the overall mean T2 relaxation time yielded complete separation between boys with DMD and healthy boys with 93.8% sensitivity and 94.4% specificity.

There was a statistically significant positive correlation between the mean FF and T2 relaxation time in all muscles (fig. 4). A significant positive correlation was found between the overall mean T2 relaxation time and age (r=0.716, P<0.001), BMI (r=0.548, P=0.001) (fig. 5), 10 m walk/run (r=0.696, P<0.001), supine up (r=0.764, P<0.001) and 4 stairs (r=0.687, P<0.001) (fig. 5). A negative correlation was also found between the overall mean T2 relaxation time and the total muscle strength score (r=−0.779, P<0.001) and NSAA (r=−0.661, P<0.001) (fig. 6).

3 DISCUSSION

In this study, we used a combination of three-point Dixon and T2 mapping to quantitatively evaluate the FF and T2 relaxation time of thigh skeletal muscles in children with DMD and healthy boys. Our data show that the FF values and T2 relaxation time in DMD

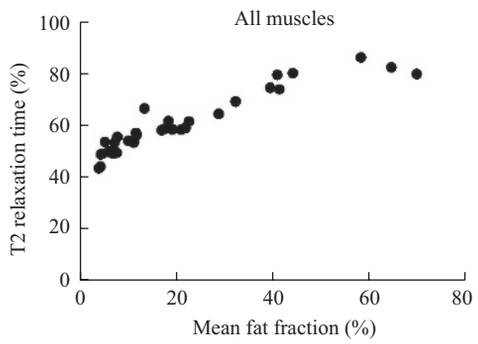

Fig. 4 Example plots showing correlation of mean fat fraction and T2 relaxation time in all muscles

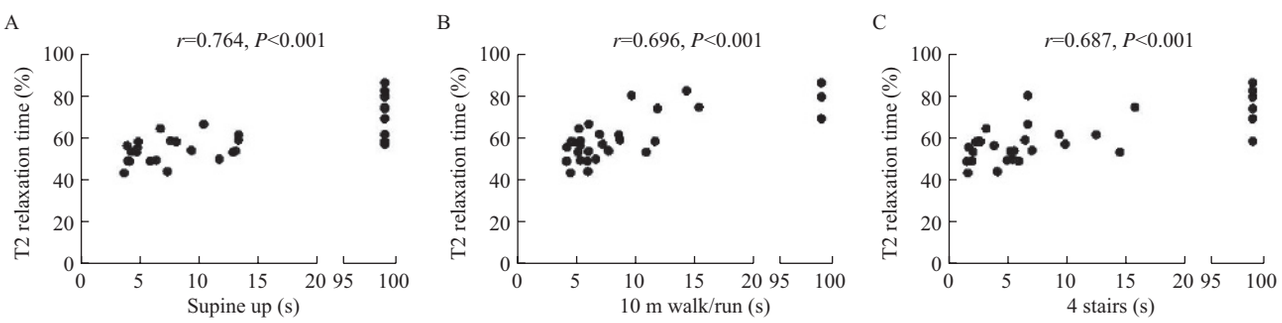

Fig. 5 Example plots showing correlation of overall mean T2 relaxation time and timed functional tests (supine up for A, 10 m walk/run for B, 4 stairs for C). For those who are unable to perform the tasks, the functional time is set to an arbitrary 99 s maximum.

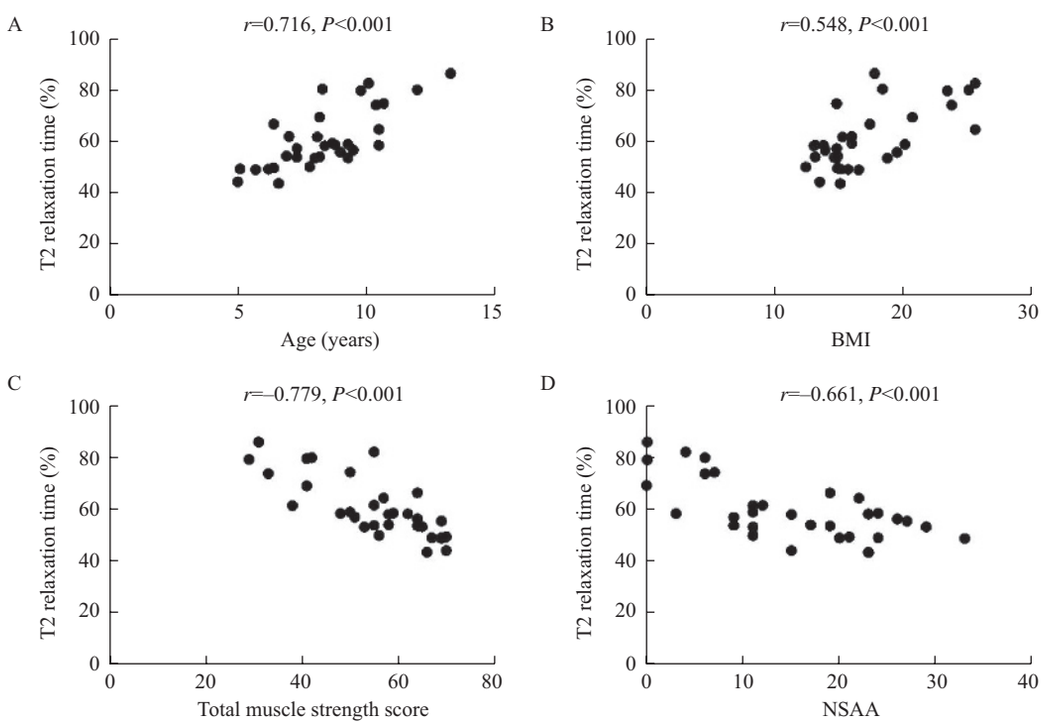

Fig. 6 Example plots showing correlation of overall mean T2 relaxation time and age (A), BMI (B), total muscle strength score (C) and NSAA (D)

patients have certain distribution pattern. In addition, the overall mean T2 relaxation time was significantly correlated with mean FF and multiple clinical assessments. Together, these results suggest that the T2 relaxation time could be an objective measure of disease severity and a useful outcome parameter in DMD trials.

The qMRI is an important non-invasive tool to quantify pathological changes and to monitor disease progression in muscular dystrophies in the skeletal muscle[25–27]. Recently, the three-point Dixon method was used *in vivo* to quantitatively calculate the FF of skeletal muscles in DMD[28–30]. T2 mapping is a quantitative measurement of T2 relaxation time[31]. Several studies have demonstrated that dystrophic muscles have longer T2 relaxation time[18, 32, 33], even in young boys with DMD[34]. Kim *et al*[35] reported that the mean T2 relaxation time of the gluteus maximus muscle in DMD patients was correlated with fatty infiltration grading assessed qualitatively on T1WI and with several clinical assessments. Moreover, T2 mapping can also be used to evaluate the longitudinal muscle changes in children who receive steroid therapy for DMD[10, 36]. However, few researchers have combined T2 mapping and IDEAL-IQ to evaluate the thigh muscles of DMD patients.

There is an increasing body of evidence showing the progressive and selective pattern of fatty infiltration in thigh muscles of DMD patients[37, 38]. Non-quantitative MRI studies suggested that the Gra, Sar, and SM muscles were relatively spared by fatty infiltration[35, 39]. Zheng *et al*[38] described a "trefoil with single fruit" sign in muscle MRI in dystrophinopathies, with relative sparing of the Gra, Sar, AL, and SM muscles. However, our results indicated that the lowest FF was in the Sar [6.9 (4.6, 12.5)%], AL [2.6 (1.5, 15.5)%], and Gra [5.1 (2.6, 8.0)%]; no significant differences were observed with the normal control. The different results were most likely the result of the fact that previous studies used a subjective method to assess the pattern of fatty infiltration and lacked a control group.

The distribution characteristics of T2 relaxation time in this study were similar to those of FF in thigh muscles. The highest mean T2 relaxation time was found in the AM (75.8±18.6 ms), whereas the lowest was found in the Gra (46.6±8.4 ms). The mean T2 relaxation time of thigh muscles in DMD group was significantly longer than that in the control group except for the Gra; these results are well consistent with those reported previously[33, 40]. However, the etiology for selective muscle involvement and relative sparing in the lower extremities of boys with DMD is still unclear. One potential consideration is that the muscles that are relatively spared may undergo reduced loading during eccentric contractions with daily activities. Indeed, several studies have reported altered gait patterns in DMD children by using quantitative gait assessment[41–43]. Elevated T2 relaxation time is usually regarded to reflect tissue oedema and inflammation. Carlier *et al*[44] recently showed that the presence of lipids generates a slowly relaxing component that shifts the overall T2 decay to potentially much longer T2s in fatty infiltrated muscles. We found the T2 relaxation time to be highly positively correlated with the mean FF values in all muscles, indicating that in DMD patients, they primarily represent fatty infiltration rather than oedema.

Clinical assessments such as the timed function tests (10 m walk/run, supine up, 4 stairs), strength assessments and NSAA used in this study are validated to assess motor impairment in muscular dystrophies in clinical trials[5, 6, 45]. A previous study showed a positive correlation between T2 relaxation time and clinical assessments, including the patient's age, clinical functional score, timed Gower score, and time to run 30 feet[35]. In this research, we found that the T2 relaxation time was positively correlated with age and BMI. This is in accordance with a study by Forbes *et al*[32] showing age-dependent increases in MRI-T2 of the lower extremity skeletal muscles. The overall mean T2 relaxation time was highly negatively correlated to clinical impairment of the lower extremities as measured by strength score and NSAA. Furthermore, T2 relaxation time was highly positively correlated with timed function tests (10 m walk/run, supine up, 4 stairs). Similarly, a recent study indicated that the global T2 relaxation time was significantly correlated with 6-min walk test, 10 m walk/run and supine up in patients with Becker muscular dystrophy (BMD)[46]. These findings show that the T2 quantification measures a key pathological process in DMD and BMD.

There were several limitations to this study. First, T2 mapping in this research was performed without fat suppression; this is a potential limitation because T2 relaxation time can reflect both increased fatty infiltration and inflammation of the muscles. The application of fat suppression has been found to enable separation of the effects of fatty infiltration on T2 relaxation time from the effects of muscle oedema[34, 47]. However, other researchers suggested that fat suppression does not always suppress the entire fat signal present in skeletal muscle; the influence of the lipid signal may not be completely ruled out from the T2 relaxation time[40, 44]. Second, this study did not take into account the possible impact of muscle activity on T2 relaxation time. Mankodi *et al*[48] demonstrated that muscle T2 increased after exercise in the DMD participants and healthy volunteers. Therefore, subjects should be asked to refrain from excessive ambulation and exercise for at least 12 h before MR

imaging[49]. Third, the IDEAL-IQ technique is rarely used in neuromuscular disorders and its accuracy and repeatability remain to be validated.

In summary, this study demonstrated that skeletal muscle FF values and T2 relaxation time of thigh muscles in DMD patients have certain distribution pattern. The T2 relaxation time was highly correlated with mean FF and multiple clinical assessments. Therefore, we conclude that quantitative T2 mapping is an accurate and non-invasive method for evaluation of disease involvement and could be a very good predictor of the functional status in DMD patients.

Conflict of Interest Statement

The authors declare that they have no conflict of interest.

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