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Quantifying disease activity in fatty-infiltrated skeletal muscle by IDEAL-CPMG in Duchenne muscular dystrophy

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Abstract

The purpose of this study was to explore the use of iterative decomposition of water and fat with echo asymmetry and least-squares estimation Carr–Purcell–Meiboom–Gill (IDEAL-CPMG) to simultaneously measure skeletal muscle apparent fat fraction and water T_2 ($T_{2,w}$) in patients with Duchenne muscular dystrophy (DMD). In twenty healthy volunteer boys and thirteen subjects with DMD, thigh muscle apparent fat fraction was measured by Dixon and IDEAL-CPMG, with the IDEAL-CPMG also providing $T_{2,w}$ as a measure of muscle inflammatory activity. A subset of subjects with DMD was followed up during a 48-week clinical study. The study was in compliance with the Patient Privacy Act and approved by the Institutional Review Board. Apparent fat fraction in the thigh muscles of subjects with DMD was significantly increased compared to healthy volunteer boys ($p < 0.001$). There was a strong correlation between Dixon and IDEAL-CPMG apparent fat fraction. Muscle $T_{2,w}$ measured by IDEAL-CPMG was independent of changes in apparent fat fraction. Muscle $T_{2,w}$ was higher in the biceps femoris and vastus lateralis muscles of subjects with DMD ($p < 0.05$). There was a strong correlation ($p < 0.004$) between apparent fat fraction in all thigh muscles and six-minute walk distance (6MWD) in subjects with DMD. IDEAL-CPMG allowed independent and simultaneous quantification of skeletal muscle fatty degeneration and disease activity in DMD. IDEAL-CPMG apparent fat fraction and $T_{2,w}$ may be useful as biomarkers in clinical trials of DMD as the technique disentangles two competing biological processes.

Keywords: Duchenne muscular dystrophy; Magnetic resonance imaging; IDEAL-CPMG; Skeletal muscle; Apparent fat fraction; Fatty degeneration; T_2 relaxation times; Water T_2

1. Introduction

Promising new treatments for Duchenne muscular dystrophy (DMD) are now being studied in clinical trials [1]. There is an urgent need for biomarkers to assess disease activity and treatment response in DMD. Since the disease pathology and treatment response may vary locally within a muscle, biomarkers that reflect the spatial distribution of the disease process within and across muscles are desired. Magnetic resonance imaging (MRI) enables non-invasive, repeatable, and objective assessment of individual muscles. In a preliminary study, muscle fat fractions quantified by Dixon correlated better with functional scores than measures of muscle strength [2]. Skeletal muscle T_2 relaxation times were described as a biomarker of disease progression [3,4] and treatment response in patients [5] and animal models of DMD [6]. However, increases in lipids and extracellular water contribute to lengthening of the apparent skeletal muscle T_2, therefore the effects of inflammatory activity cannot be distinguished from fatty infiltration. Absence of muscle fat in mouse models makes apparent T_2 and water T_2 measurements almost equivalent [7]. In contrast increases in apparent T_2 in patient muscle are proportional to increase in muscle fat fraction, and provide little information on inflammatory activity [7–9]. Simultaneous measurements of T_2-weighted fat and T_2-weighted water images independently within a single acquisition make iterative decomposition of water and fat with echo asymmetry and least-squares estimation Carr–Purcell–Meiboom–Gill (IDEAL-CPMG) an attractive MRI technique for measuring inflammatory disease activity in the presence of fatty degeneration [7,10]. The present study explored the use of

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IDEAL-CPMG in assessing disease activity in subjects with DMD participating in a clinical trial.

2. Materials and methods

2.1. Participants and study design

The DMD Imaging Study was a 48-week prospective imaging study conducted between January 2012 and October 2013 as an optional sub-study of a multi-center phase 2, double blind, placebo-controlled trial of oligonucleotide therapy in ambulatory boys with DMD resulting from a mutation potentially corrected by skipping exon 51 (DMD114876; NCT01462292). The DMD imaging study was offered to all subjects eligible for the DMD114876 study before or during their first screening visit. Appendix E1 (online) contains details of subject eligibility, recruitment, and inclusion and exclusion criteria. Subjects in the larger study received either drisapersen (3 or 6 mg/kg/week) or placebo for 24 weeks followed by a 24-week post-treatment phase. The subjects traveled to our Clinical Center during the screening phase, and at 12, 24, and 48 weeks. Subjects not eligible for randomization in the clinical trial had a one-time evaluation during the screening phase. Pilot data were also obtained from age-range matched healthy volunteer (HV) boys who were recruited from our Clinical Research Volunteer Program registry. All subjects were asked to avoid any excessive physical activity beyond their normal levels for a week prior to each study visit.

2.2. Standard protocol approvals, registrations, and patient consents

The study was registered on clinicaltrials.gov (NCT01451281) and was in compliance with the Patient Privacy Act and approved by the Institutional Review Board. Informed written assent and consent were obtained from the subject and parent or guardian before participation in the study.

2.3. MRI acquisition

MR images included a T_1-weighted scan, an IDEAL-CPMG scan, and a DIXON scan acquired on a 3T Verio scanner (Siemens, Erlangen, Germany) at the Clinical Center. A Siemens 8 channel Body Matrix Coil Array was used for the thighs. All scans were performed on the same scanner. A parent or staff member was present in the scanner suite to alleviate subject anxiety. MR operators were trained to ensure consistency in the image acquisition across study visits.

Imaging was performed at mid-thigh, defined on the right femur as the midpoint between the inferior edge of the right medial femoral condyle and the inferior edge of the right femoral head. T_1-weighted turbo spin echo images were acquired consisting of 21 5 mm slices with 5 mm gap and 1.5 mm in-plane resolution. The IDEAL-CPMG [10] acquisition measured 3 gradient echoes around each of $N = 12$ spin echo times (10 ms, 20 ms, . . .,120 ms). The gradient echoes sampled three fat-water shifts at ($-5\pi/6$, $\pi/2$, $11\pi/6$) [11] around each spin-echo time. Sixteen 5 mm slices with 5 mm gap were imaged in a 40×20 cm FOV with matrix size of 160×80, resulting in $2.5 \times 2.5 \times 5$ mm voxel size with a TR of 5 s, resulting in a scan time of 6 m: 45 s. The Dixon acquisition consisted of 2 gradient echoes at 2.45 and 3.675 ms (nominally in-phase and out-of-phase at 3T) with a B0 field map in a 24 cm 3D slab with 48 5 mm sections in a 36×25 cm FOV with 256×256 matrix size, resulting in $1.4 \times 1.4 \times 5$ mm voxels. The Dixon scan used a TR of 5.64 ms, flip angle 9°, and 2 averages in each of two abutting 3D slabs, resulting in 1 m: 40 s scan time.

2.4. MRI analysis

The T_1-weighted scans were used as an anatomical reference image for the co-registration of all other within-visit scans for a given subject and for definition of the six thigh muscle regions-of-interest (ROIs): bicep femoris, rectus femoris, semitendinosus, vastus intermedius, vastus lateralis, and vastus medialis in the central five imaging slices of the right leg covering a distance of 50 mm. Definition of these muscle groups was performed manually using the Analyze 11.0 software package (AnalyzeDirect, Overland Park, KS). The co-registration process was a rigid-body (six degrees of freedom) transformation using the FSL tool FLIRT [12]. For two scans (one baseline and one 24-week scan), the T_1-weighted image was incomplete/unusable, so the in-phase Dixon scan was instead used as the anatomical reference image.

Dixon images were reconstructed using Siemens (Erlangen, Germany) product software producing a single water image (s_w) and fat image (s_f) generated from two gradient echo images and a field map [13]. The apparent fat fraction (*AFF*) measured using Dixon was then calculated as $AFF_{Dixon} = 100 * |s_f| / (|s_f| + |s_w|)$.

IDEAL-CPMG was reconstructed using in-house MATLAB code to generate a $n = 1 \ldots N$ series of T_2-weighted water images, $\rho_w[n]$, and T_2-weighted fat images, $\rho_f[n]$. The water proton density ($\rho_{0,w}$), fat proton density ($\rho_{0,f}$), T_2 of water ($T_{2,w}$), T_2 of fat ($T_{2,f}$), were then fit according to $\rho_w[n] = \rho_{0,w} \exp(-10ms \cdot n/T_{2,w}) + N_w$ and $\rho_f[n] = \rho_{0,f} \exp(-10ms \cdot n/T_{2,f}) + N_f$ where N_w and N_f account for the non-zero floor due to sequence imperfections (e.g. B1-inhomogeneity, stimulated echoes) and the noise floor of magnitude data [14]. The T_2-corrected *AFF* measured using IDEAL-CPMG was then calculated as $AFF_{IDEAL-CPMG} = 100 * |\rho_{0,f}| / (|\rho_{0,f}| + |\rho_{0,w}|)$. Full details of the reconstruction and fitting can be found elsewhere [10].

For both the Dixon and the IDEAL-CPMG sequences, the reconstructed in-phase image was used for co-registration to the corresponding T_1-weighted scan, with the computed co-registration parameters subsequently used to transform all of the remaining maps for that given imaging sequence (i.e., the T_2 maps ($T_{2,w}$, $T_{2,f}$) and *AFF* maps for IDEAL-CPMG, and the *AFF* maps for Dixon). For each of the processed maps the mean of the signal in each of the muscle ROIs was generated using in-house MATLAB functions. All investigators were blinded to subject grouping and treatment during analysis.

2.5. Statistical analysis

A two-sample *t*-test, nonparametric (Mann–Whitney) test, and Fisher's z transformation of sample correlation coefficients were used in cross-sectional comparisons. The level of

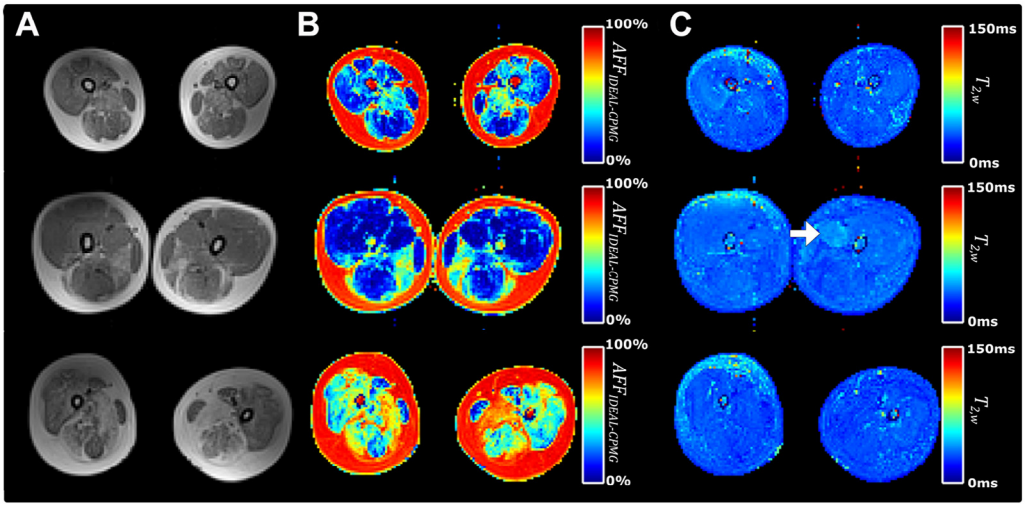

Fig. 1. Representative T_1-weighted and IDEAL-CPMG images of the thigh muscles in three subjects with Duchenne muscular dystrophy (DMD). A T_1-weighted image (A), T_2-corrected fat fraction map (B), and water-T_2 map (C) are shown representing subject anatomy, changes in muscle apparent fat fraction ($AFF_{IDEAL-CPMG}$) and muscle water T_2 ($T_{2,w\ IDEAL-CPMG}$) respectively in the thigh muscles of subjects with DMD. A different severity of fatty degeneration is present in the thigh muscles of each subject, whereas inflammatory activity is sparse and seen in only few muscles (arrow).

significance was set at $p < 0.05$ without corrections for multiple comparisons. The degree of agreement was measured by the Bland–Altman method [15]. Pearson's correlation was used for continuous variables. Spearman's rank correlation was used for ordinal variables. Data analyses were done using Prism 6.0 (Graphpad Software, La Jolla, CA, USA).

3. Results

3.1. Subject characteristics

Baseline MRI data were obtained from 13 boys with DMD and 20 HV boys. Both groups were similar in age (DMD: 6–14 years; mean ± SD: 8.8 ± 2.4 years; and HV: 5–14 years; mean ± SD: 10 ± 2.1 years; $p = 0.13$) and age-adjusted height ($p = 0.06$) and weight ($p = 0.17$). Nine subjects with DMD, who were randomized to drisapersen 3 mg/kg/week (n = 6) or placebo (n = 3), returned for follow up visits at 12, 24, and 48 weeks during the DMD114876 trial. Three subjects did not have follow up visits because they were not randomized in the clinical trial, and one subject withdrew from the study after the baseline visit due to family/personal reasons.

3.2. Thigh muscle apparent fat fraction (AFF) and water T_2 ($T_{2,w}$) in age-range matched healthy volunteers and subjects with Duchenne muscular dystrophy (DMD)

Fig. 1 shows a T_1-weighted image and IDEAL-CPMG images including T_2-corrected *AFF* map and $T_{2,w}$ map from representative slices in three subjects with DMD. A variable degree of fatty replacement is seen in all subjects, but edema is less prominent and seen in isolated muscle groups in a subset of subjects (white arrow) on the $T_{2,w}$ map.

The *AFF* in six thigh muscles measured by IDEAL-CPMG and Dixon was about 2 to 4 fold higher in subjects with DMD than the age-range matched HV boys (Fig. 2A and B, Table 1). A linear regression model using factor status, HV or DMD, and age as a covariate showed a significantly higher *AFF* in subjects with DMD compared to HV boys at all ages ($p \leq 0.001$). The range of Dixon *AFF* in the thigh muscles was 4%–17% in HV boys and 8%–75% in subjects with DMD and that of IDEAL-CPMG *AFF* was 3%–20% in HV boys and 7%–84% in subjects with DMD (Table 1). There was a strong correlation between IDEAL-CPMG and Dixon for *AFF* measurements in the same muscle (n = 180 muscles, Spearman $r = 0.92$; $p < 0.0001$). The

Table 1
MRI measures of muscle apparent fat fraction (*AFF*) in the thigh muscles of age-range matched healthy volunteers (HV) and subjects with Duchenne muscular dystrophy (DMD).

Muscles	IDEAL-CPMG Median *AFF* (%) (range)			Dixon Median *AFF* (%) (range)		
	HV (n = 14)	DMD (n = 10)	*p* value Mann–Whitney	HV (n = 16)	DMD (n = 8)	*p* value Mann–Whitney
Biceps femoris	7.4 (4.2–17.5)	23.5 (16.4–80.8)	<0.0001	10.5 (5.1–15.8)	23.0 (17.3–74.7)	<0.0001
Rectus femoris	6.5 (4.1–20.3)	28.2 (11.8–84.0)	<0.0001	6.6 (3.6–11.7)	20.2 (13.4–71.7)	<0.0001
Semitendinosus	8.3 (4.1–19.6)	19.1 (11.8–49.9)	<0.001	8.1 (5.1–13.3)	15 (13.6–40.2)	<0.0001
Vastus intermedius	3.6 (3–10.1)	15.1 (7.3–79.4)	<0.001	7 (4.8–10.7)	15.5 (8.5–74)	<0.0001
Vastus lateralis	5.2 (3.9–17.5)	18.3 (8.3–83.9)	<0.001	11 (5.7–17.4)	18.8 (11.4–75.4)	<0.001
Vastus medialis	5 (3.8–19.7)	17.9 (8.0–80.5)	<0.001	6.7 (4.8–11.4)	14.1 (9.9–72.3)	<0.0001

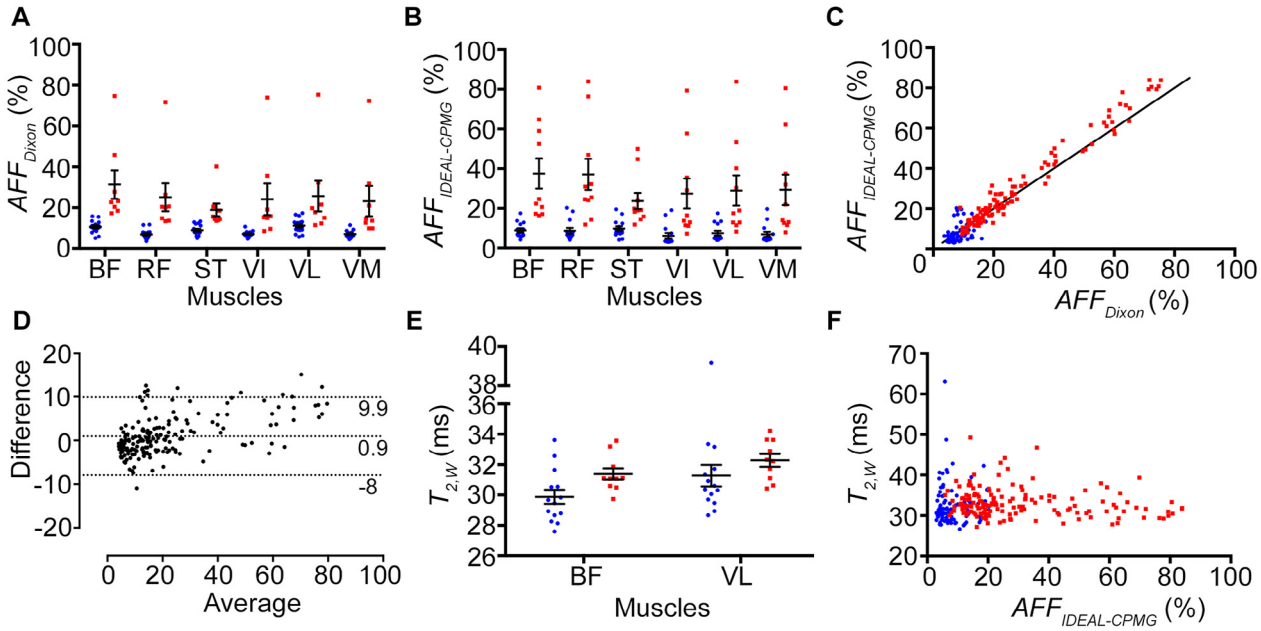

Fig. 2. MRI quantification of thigh muscle apparent fat fraction (*AFF*) and water T_2 ($T_{2,w}$) in age range-matched healthy volunteers (HV) and subjects with Duchenne muscular dystrophy (DMD). (A,B) Comparison of *AFF* measured by Dixon (A) and IDEAL-CPMG (B) in the biceps femoris (BF), rectus femoris (RF), semitendinosus (ST), vastus intermedius (VI), vastus lateralis (VL), and vastus medialis (VM) muscles of HV boys (blue circles) and subjects with DMD (red squares). Mean *AFF* measured by both methods was significantly higher in all the muscles in subjects with DMD relative to HV ($p < 0.001$). (C,D) Line of identity (C) and Bland–Altman plot (D) demonstrate excellent agreement between the two methods. Difference = $AFF_{IDEAL\text{-}CPMG} - AFF_{Dixon}$. Dotted lines in D represent mean difference and limits of agreement (mean ± 1.96 S.D.) (E) Mean $T_{2,w}$ values were significantly higher in the BF ($p = 0.02$) and VL ($p = 0.03$) of subjects with DMD (red squares) relative to HV boys (blue circles). (F) Graph shows that $T_{2,w}$ measurements were independent of the variation in *AFF* in 240 muscles examined in HV boys (blue circles) and subjects with DMD (red squares). Error bars represented as mean ± SEM.

mean difference, IDEAL-CPMG minus Dixon, was 0.95 (95% CI 0.29, 1.63). Fig. 2C and D show agreement between the two methods. Bland and Altman plot shows that 95% of the differences in the *AFF* were within the limits of agreement (−7.97, 9.88) (Fig. 2D). We found significant correlations among all muscles of subjects with DMD for IDEAL-CPMG and Dixon *AFF* (Table 2).

The IDEAL-CPMG $T_{2,w}$ of the biceps femoris (mean ± S.E.M 31.4 ± 0.4 v. 29.9 ± 0.5; $p = 0.02$) and vastus lateralis (mean ± S.E.M 32.3 ± 0.4 v. 31.3 ± 0.7; $p = 0.03$) muscles was significantly higher in subjects with DMD than HV boys (Fig. 2E, Table 3). The $T_{2,w}$ of other muscles was not significantly different between the two groups. There was no correlation between IDEAL-CPMG $T_{2,w}$ and *AFF* in the same muscle (n = 240 muscles, Spearman $r = 0.04$; $p = 0.48$) (Fig. 2F).

3.3. Age correlations with thigh muscle IDEAL-CPMG AFF and $T_{2,w}$ in subjects with DMD

The *AFF* of the biceps femoris, vastus medialis, vastus intermedius and vastus lateralis muscles increased with age in subjects with DMD (Pearson $r = 0.65$–0.69; $p < 0.05$) (Fig. 3A). There was no significant correlation between age and *AFF* in the rectus femoris and semitendinosus muscles (Pearson $r = 0.53$–0.54; $p = 0.1$). The $T_{2,w}$ decreased with age in the rectus femoris muscle (Pearson $r = -0.67$, $p < 0.05$), whereas there was no significant correlation in the other muscles (Pearson $r = -0.56$–0.04; $p = 0.09$–0.4) of subjects with DMD (Fig. 3B). No correlations were found between age and the muscle *AFF* or $T_{2,w}$ in HV boys.

MRI scans were evaluated in 4 subjects with DMD randomized to drisapersen at 3 mg/kg/week (age range: 7–10 years) and 2 subjects (age: 6 years) randomized to placebo at baseline, 24 weeks, and 48 weeks during the DMD114876 trial.

Table 2
Spearman correlations between muscle $AFF_{IDEAL\text{-}CPMG}$ (a) and AFF_{Dixon} (b) in six thigh muscles of subjects with Duchenne muscular dystrophy (DMD).

(a)

Muscles	BF	RF	ST	VI	VL	VM
BF	1	0.73*	0.74*	1****	0.94***	0.91***
RF		1	0.65	0.73*	0.81**	0.81**
ST			1	0.74*	0.83**	0.88**
VI				1	0.94***	0.91***
VL					1	0.93***
VM						1

(b)

Muscles	BF	RF	ST	VI	VL	VM
BF	1	0.90*	0.69	0.90**	0.90**	0.90**
RF		1	0.40	0.76*	0.71	0.71
ST			1	0.79*	0.74	0.74
VI				1	0.95**	0.95**
VL					1	1***
VM						1

* $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$; **** $p < 0.0001$.
BF = biceps femoris, RF = rectus femoris, ST = semitendinosus, VI = vastus intermedius, VL = vastus lateralis, VM = vastus medialis.

Table 3
MRI measures of water T_2 ($T_{2,w}$) in the thigh muscles of age-range matched healthy volunteers (HV) and subjects with Duchenne muscular dystrophy (DMD).

Muscles	IDEAL-CPMG Median muscle $T_{2,w}$ (ms) (range)		
	HV (n = 14)	DMD (n = 10)	p value Mann–Whitney
Biceps femoris	29.6 (27.6–33.6)	31.1 (29.7–33.6)	0.02*
Rectus femoris	38.8 (28.2–63.1)	34.5 (29.4–44.2)	0.6
Semitendinosus	28.3 (26.7–32.6)	29.6 (28.1–34.3)	0.2
Vastus intermedius	31.1 (28.9–40.6)	31.7 (30.0–33.5)	0.1
Vastus lateralis	30.5 (28.7–39.2)	32.3 (30.4–34.2)	0.03*
Vastus medialis	34.8 (30.3–39.5)	32.8 (30.1–38.1)	0.1

* $p < 0.05$.

Images in the remaining 3 subjects were not suitable for quantitative longitudinal analysis due to motion artifacts or fat/water swaps across ROIs. The *AFF* of the thigh muscles ranged from 45% to 76% in the 10 year old subject and ≤ 25% in the younger subjects. The six year-old subjects (n = 2) in the placebo group had ≤ 2% increase in mean muscle *AFF*, whereas the 7–10 year old subjects (n = 4) assigned to treatment group had ≥ 6% increase in mean muscle *AFF* of the thigh muscles during the 48-week follow up. The $T_{2,w}$ range (29–44 ms) in the thigh muscles was not different between the subjects in the placebo and treatment groups. There was no apparent effect of the treatment on *AFF* or $T_{2,w}$ in this cohort.

3.4. Correlations between six-minute walk distance and thigh muscle $AFF_{IDEAL\text{-}CPMG}$ in subjects with DMD

All subjects showed a change in the six-minute walk distance (6MWD) over the 48-week period (Fig. 4A). The six and seven year-old subjects had an increase in the 6MWD, whereas DMD subjects age >7 years had a decline in the 6MWD, as shown previously [16]. There was an exception in that the seven year-old subject had a lower 6MWD relative to the eight year-old subject throughout the study period. Notably, the seven year-old subject had a higher *AFF* in all six muscles compared to the eight-year old subject. We observed that changes in *AFF* of the thigh muscles over time had a trend opposite to that of the 6MWD in younger vs. older subjects with DMD (Fig. 4B; vastus lateralis muscle shown). We examined correlations between all available paired measurements of *AFF* in individual thigh muscles and 6MWD in subjects with DMD (n = 22 for each muscle; each pair was treated independently). Strong negative correlations were found between the 6MWD and *AFF* in all thigh muscles of subjects with DMD examined in this study (Spearman $r = -0.75$ to -0.57; $p \leq 0.004$) (Fig. 4C; vastus lateralis muscle shown). Subjects with ≤ 20% *AFF* in the vastus lateralis muscle walked > 400 m and conversely, those with > 20% *AFF* in the vastus lateralis muscle walked < 400 m in 6 minutes.

The progression of muscle *AFF* (as in Ref. [17]) and muscle water T_2 was different in individual thigh muscles followed over the 48-week period (Fig. 4D and E). In agreement with a recent publication [18], we found that the IDEAL-CPMG sequence detected modest increases in the thigh muscles *AFF* in the six and seven year-old subjects whose 6MWD improved over the year (Fig. 4D). The greatest increase (>10%) in the muscle *AFF* was found in the biceps femoris and vastus lateralis muscles of the eight and nine year-old subjects whose 6MWD declined by >30 m (Fig. 4E). Overall, there was a greater increase in the muscle *AFF* in the older subjects whose 6MWD decreased compared to the younger subjects whose 6MWD increased during the 48-week follow up period. Conversely, the changes in muscle water T_2 were more remarkable in the younger subjects with increased 6MWD compared to the older subjects with decreased 6MWD over the year (Fig. 4D and E).

4. Discussion

Quantitative MRI correlates of fatty degeneration and muscle inflammatory activity are potentially useful for monitoring disease progression and treatment response during a clinical trial. The MRI parameter of muscle inflammatory activity corresponds to water mobility within muscle tissue ($T_{2,w}$) and may reflect loss of muscle fiber integrity in the dystrophic lesions and in the areas of inflammation and necrosis in skeletal muscle. Changes in $T_{2,w}$ are dynamic and sensitive to the underlying disease activity. Fatty replacement of muscle tissue occurs during later stages in the disease process. Dixon separates muscle fat and water based on phase difference (chemical shift) and provides measurement of muscle fat without any confounding effect of muscle edema. However, quantification of muscle inflammatory activity in fat-infiltrated dystrophic muscles has been challenging because increases in muscle water T_2 are masked by the presence of muscle fat [7,9]. We present IDEAL-CPMG as an MRI biomarker for simultaneous quantification of skeletal muscle $T_{2,w}$ and *AFF* in DMD. Our results show that IDEAL-CPMG reliably and efficiently separates fat and water components of T_2 in the thigh muscles, which are affected early and more severely than other muscles in subjects with DMD. There was no relationship between $T_{2,w}$ and *AFF* ($r = 0.04$), indicating that there is little fat contamination within the IDEAL-CPMG water signal and that the level of inflammatory activity does not change with increases in *AFF* in the thigh muscles of subjects with DMD. This agrees with previous studies in DMD using spectroscopy in the lower leg muscles [3] and the forearm muscles [19] but contrasts to previous studies in hypokalemic periodic paralysis [20] where a weak correlation ($r = 0.54$) between $T_{2,w}$ and *AFF* in the lower leg muscles was observed.

MR spectroscopy (MRS) is the standard for noninvasive measurement of muscle fat. However, poor spatial resolution and coverage as well as challenges in the spectroscopy volume registrations consistently between imaging visits limit its use in multicenter clinical studies. IDEAL-CPMG and Dixon provide better spatial resolution and coverage, enabling assessment of multiple muscles simultaneously [10,21–23]. This is pertinent in DMD because the disease varies locally within a muscle and between muscles in patients. Variations of Dixon have been used in *AFF* quantification in DMD [2,22,24,25]. We observed a strong correlation (r = 0.92) between IDEAL-CPMG and Dixon across a broad range (3%–84%) of muscle fat values. The Dixon sequence is T_1-weighted, with the level of fat

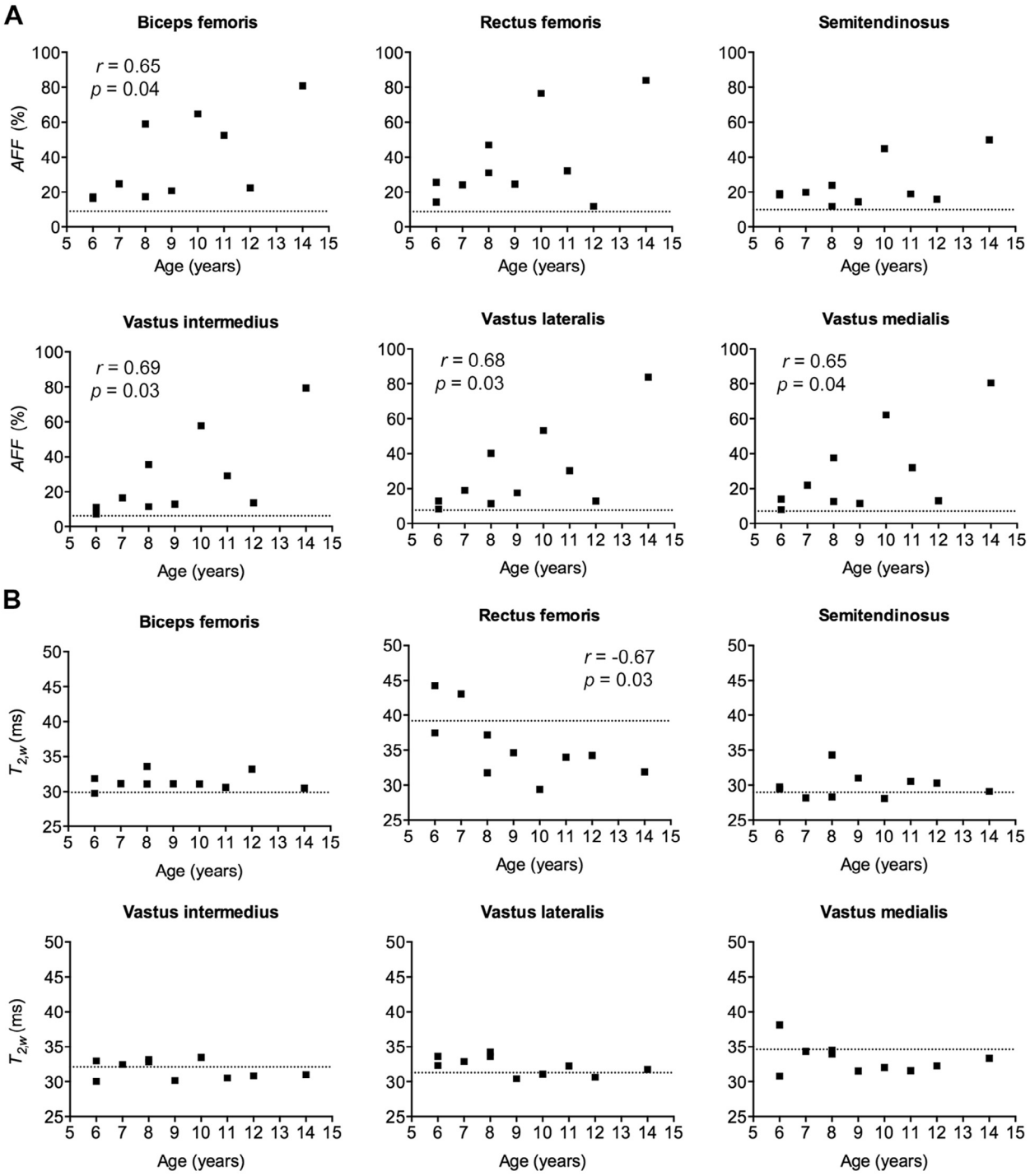

Fig. 3. Age correlations with thigh muscle IDEAL-CPMG apparent fat fraction (*AFF*) and water T_2 ($T_{2,w}$) in subjects with Duchenne muscular dystrophy (DMD). (A) There is a significant increase in *AFF* of the biceps femoris, vastus intermedius, vastus lateralis and vastus medialis muscles with age. (B) Conversely, there is a significant decrease in $T_{2,w}$ of the rectus femoris muscle with age. Pearson correlation coefficient (*r*) *is* shown. Dotted lines represent mean values of the age-range matched HV boys.

overestimated from the shorter T_1 of fat versus water. The IDEAL-CPMG sequence did not suffer from T_1 saturation, but a magnetization transfer effect from the radiofrequency pulses would reduce only the water signal. Thus, both methods suffer from reduction of the water signal, but for different reasons. Further, the IDEAL-CPMG decomposition used a 7-peak fat multispectral model [10], whereas the Dixon formulation assumes a single fat peak. The implicit assumption of a single T2* value in the Dixon sequence may explain the increased difference between IDEAL-CPMG and Dixon at high fat fractions [26].

The *AFF* and $T_{2,w}$ changes had a different muscle pattern and evolution with age in subjects with DMD. Fatty degeneration

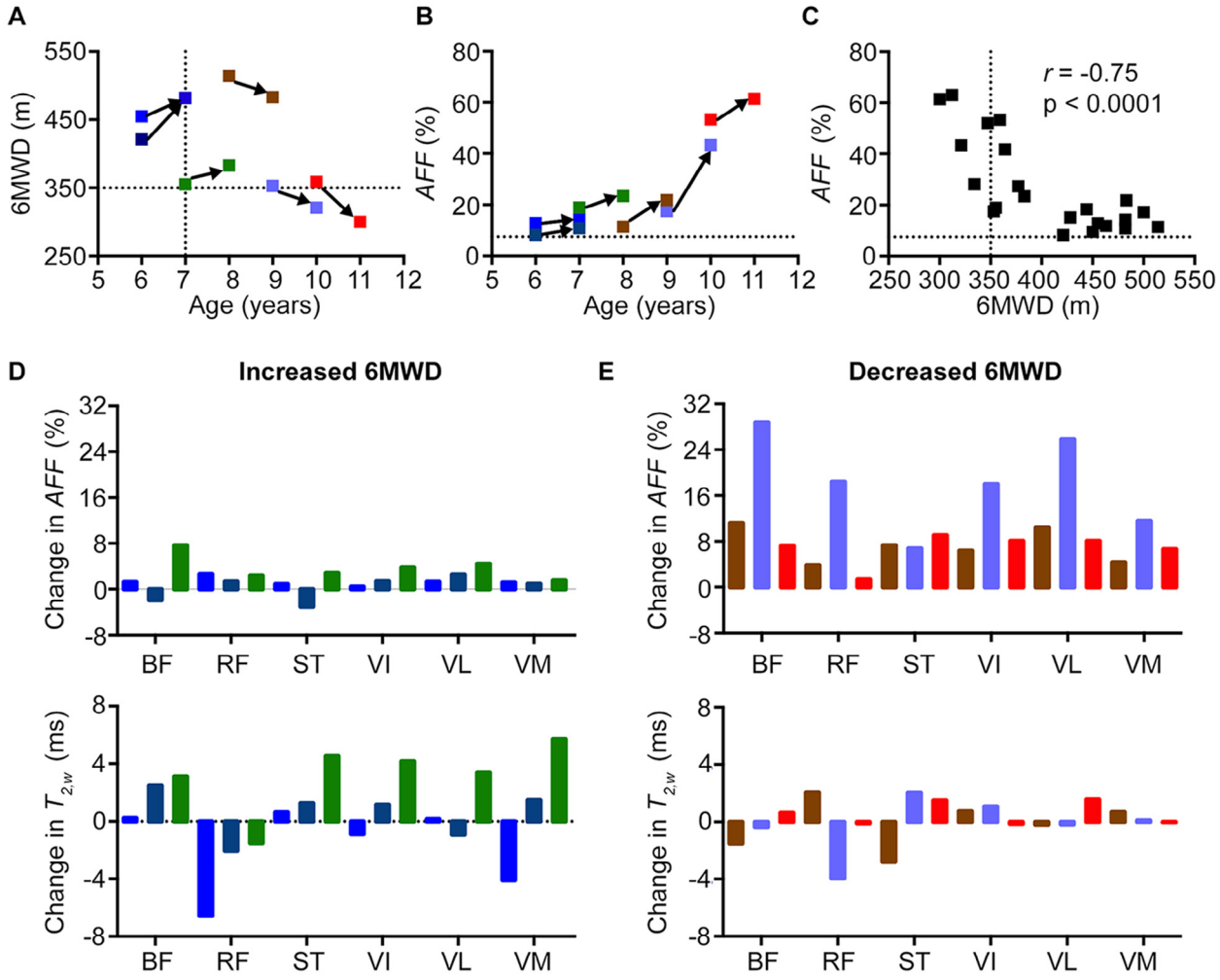

Fig. 4. Change in the six-minute walk distance (6MWD) and thigh muscle IDEAL-CPMG apparent fat fraction (*AFF*) and water T_2 ($T_{2,w}$) in subjects with Duchenne muscular dystrophy (DMD) from baseline to 48 weeks (including 24-week treatment phase plus 24-week follow up) in the clinical trial. (A,B) Change in the 6MWD and the vastus lateralis muscle *AFF* from baseline to 48 weeks. The 6MWD increased in the six (light or dark blue) and seven (green) year-old subjects and decreased in the eight (brown), nine (purple) and 10 (red) year-old subjects (A). Dotted lines represent predictor cutoffs of decline in ambulation (age > 7 years and 6MWD < 350 m) [16]. The vastus lateralis muscle *AFF* increased in all subjects (B). Dotted line represents the mean *AFF* of the age-range matched HV boys. Time scale is shown from individual subject's age at baseline. (C) Correlation between paired measurements of the 6MWD and the vastus lateralis muscle *AFF*. Spearman *r* is shown. Dotted horizontal line represents mean *AFF* of the age-range matched HV boys (7.5%) and dotted vertical line represents predictor cutoff of decline in ambulation (6MWD <350 m). (D,E) Change in muscle *AFF* and $T_{2,w}$ of the biceps femoris (BF), rectus femoris (RF), semitendinosus (ST), vastus intermedius (VI), vastus lateralis (VL) and vastus medialis (VM) muscles of subjects with increased 6MWD (D) and decreased 6MWD (E). Each bar represents an individual subject with color schema as in A and B.

was prominent and dystrophic inflammation or edema was relatively sparse in the thigh muscles of the DMD cohort with 11 of 13 subjects ≥ 7 years of age. The biceps femoris and vastus lateralis muscles were most responsive to disease progression consistent with previous observations [3,18]. The change in the $T_{2,w}$ of the thigh muscles was relatively less in the >7 year-old subjects compared to younger ones. These findings support previous observations that MRS $T_{2,w}$ may be a sensitive marker of response to prednisone before skeletal muscles are loaded with fatty degeneration [5] and that MRS $T_{2,w}$ tends to normalize with disease progression in DMD [3,19]. The $T_{2,w}$ was reliably measured in the hamstrings and vastus muscles (SE ±0.5 ms to ±0.8 ms). However, B1 inhomogeneity due to the relatively high contribution from stimulated echoes within the rectus femoris muscle precluded accurate $T_{2,w}$ assessment (SE ±2.4 ms). This could be addressed by using extended phase graph fitting as proposed by Lebel and Wilman [27,28] instead of the monoexponential least squares fitting employed in this study. Other methodological limitations include a restricted spatial resolution demanded by time constraints in a pediatric study, and a simplistic fitting model for T_2 decay in magnitude images [29]. Further studies are needed to more fully qualify these biomarkers, including determining the inter- and intra-rater and site variability.

We did not see a therapeutic response to drisapersen on MRI measures of fatty infiltration and edema in this exploratory imaging substudy, probably because (1) few subjects participated, (2) subjects receiving placebo and active agent had

different age distributions due to randomization and (3) no subjects received the higher dose (6 mg/kg/week) found to be efficacious elsewhere [30], again due to randomization.

Our findings support the conclusions of earlier studies [18,31] that MRI measures are sensitive to disease progression that cannot be identified by functional testing such as 6MWD. We found that subjects with DMD who walked about 350 m at baseline had a broad range of *AFF* in each muscle. The 6MWD and other functional assessments are influenced by patient motivation and cooperation, which may be challenging in a pediatric population. Furthermore, multiple muscle groups differently affected by the disease are recruited during the activity. In contrast, MRI biomarkers provide objective assessments of individual muscles to monitor disease progression and treatment response during clinical trial. Skeletal muscle *AFF* at baseline in subjects with DMD may serve as an objective indicator of disease severity and help to select or stratify subjects appropriately for clinical trials.

In summary, IDEAL-CPMG provides independent and simultaneous quantification of disease activity and fatty degeneration in multiple skeletal muscles of subjects with DMD. The $T_{2,w}$ changes are sparse in the thigh muscles of this age group in DMD, agreeing with MRS [3,19] and in contrast with apparent muscle T_2 relaxation times [3,4]. IDEAL-CPMG and Dixon agree well on *AFF* measurements. IDEAL-CPMG *AFF* and $T_{2,w}$ may be useful as an imaging biomarker in future trials.

Authors' contributions

Design and conceptualization of the study (AM, KF, RJ), data analysis and interpretation (AM, CB, RN, RJ), statistical analysis (AM, SA), drafting the manuscript and revising the manuscript for intellectual content (AM, RJ, CB, RN, KF). AM had access to all the data and takes responsibility for the data, accuracy of the data analysis, and the conduct of the research.

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Appendix: Supplementary material

Supplementary data to this article can be found online at doi:10.1016/j.nmd.2016.07.013.

Abbreviations

IDEAL-CPMG	iterative decomposition of water and fat with echo asymmetry and least-squares estimation Carr–Purcell–Meiboom–Gill
AFF	apparent fat fraction
$T_{2,w}$	water T_2
DMD	Duchenne muscular dystrophy
MRI	magnetic resonance imaging
MRS	magnetic resonance spectroscopy
ROI	region of interest
6MWD	six-minute walk distance

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