

# Three-Point Technique of Fat Quantification of Muscle Tissue as a Marker of Disease Progression in Duchenne Muscular Dystrophy: Preliminary Study

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**OBJECTIVE.** Clinical trials involving patients with Duchenne muscular dystrophy are hindered by the lack of suitable objective end points. The purpose of this study was to examine whether muscle lipid infiltration measured with the three-point Dixon MRI technique has value as a marker of disease severity.

**SUBJECTS AND METHODS.** Disease severity in nine boys (mean age,  $8.6 \pm 2.7$  years) with Duchenne muscular dystrophy was determined with the functional ability scale of Brooke and associates. Functional scores were compared with strength measurements obtained by manual testing of muscles of the lower extremities, knee extensor strength measured with an isokinetic dynamometer, and muscle fat percentage in the quadriceps and hamstrings determined with the three-point Dixon MRI technique.

**RESULTS.** MRI measurements of fat infiltration had stronger correlation ( $p < 0.05$ ) with functional grade than did measurements obtained with manual muscle testing ( $p = 0.07$ ) or quantitative strength measured with the isokinetic dynamometer ( $p = 0.54$ ). Muscle fat percentage did not correlate with strength measurements from manual or dynamometer muscle testing but increased with age in subjects with Duchenne muscular dystrophy.

**CONCLUSION.** Muscle adiposity values obtained with three-point Dixon MRI are accurate in assessment of disease severity in patients with Duchenne muscular dystrophy. Because they are not influenced by patient effort or examiner variability, these measurements are more objective and reproducible than measurements of muscle strength.

**D**uchenne muscular dystrophy (DMD) is one of the most common inherited degenerative diseases of skeletal muscle, affecting one in 3,500 boys. The disease is caused by mutations in the X-linked dystrophin gene [1]. Patients present with weakness by the age of 5 years, are usually using a wheelchair in their early teens, and die of cardiopulmonary complications in their teens or twenties. There is neither a cure nor recognized therapy for slowing muscle wasting [2–4].

The overall strength, rate of decline in strength, and functional ability of DMD patients of the same age vary greatly, making a reliable prognosis for duration of ambulation difficult [5–7]. The lack of proven therapies for DMD is due in part to the heterogeneity of the disease and to the lack of standardized and validated approaches to assessment of disease activity and muscle damage. Current outcome measures, or surrogate markers, of muscle injury in patients with DMD, that is, functional mobility, muscle strength, and blood levels of creatine phosphoki-

nase, are not adequate for longitudinal studies or treatment assessment. Muscle biopsy, which has long been recognized as the reference standard for monitoring disease and interventions, is invasive and difficult to justify [8–14]. Therefore, much emphasis is being placed on developing objective noninvasive markers that can be used as predictors of disease severity.

Several investigators [11, 15–18] have used MRI as a possible adjunct to physical examination and as a means for further exploring the increase in fat tissue in dystrophic muscle in boys with DMD. In only one of these studies [11], however, did investigators compare MRI measurements of fat infiltration in muscle with results of standard grading of functional ability. In none of the studies were MRI measurements compared with measurements of muscle strength. Previous studies also were limited by the use of conventional MRI, in which the signal intensity within a voxel (the unit of measurement) is the vector sum of the fat and water signal intensities of the protons within that voxel.

**Keywords:** fat quantification, MRI, muscle, muscular dystrophy, three-point Dixon technique

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## MRI of Duchenne Muscular Dystrophy

The three-point Dixon MRI technique is a powerful way to quantify the individual contributions of fat and water in each voxel of tissue, from which the fat fraction is calculated for detection of signal intensity from small numbers of fat protons [19]. In this approach, the chemical shift difference between water and fat is encoded into images with different echo shifts [20, 21]. The purpose of this study was to examine the usefulness of the three-point Dixon MRI technique in quantifying muscle fat infiltration as a marker of disease progression in boys with DMD. Dixon MRI measurements were compared with traditional strength measurements obtained with manual muscle testing and quantitative strength testing with an isokinetic dynamometer.

### Subjects and Methods

The study subjects were nine boys (mean age,  $8.6 \pm 2.7$  years; range, 4–13 years) with DMD. The diagnosis of DMD was confirmed with DNA testing, biopsy, or both. The subjects had no history of chronic illness other than DMD (including any neuromuscular, metabolic, or endocrine disorder that could alter bone or muscle metabolism) and were able to cooperate and participate in the various tests. The investigational protocol was approved by the institutional review board for clinical investigations at our institution, and informed consent was obtained from all parents. The MRI and functional and strength measurements were performed by different investigators, who were blinded to one another's results.

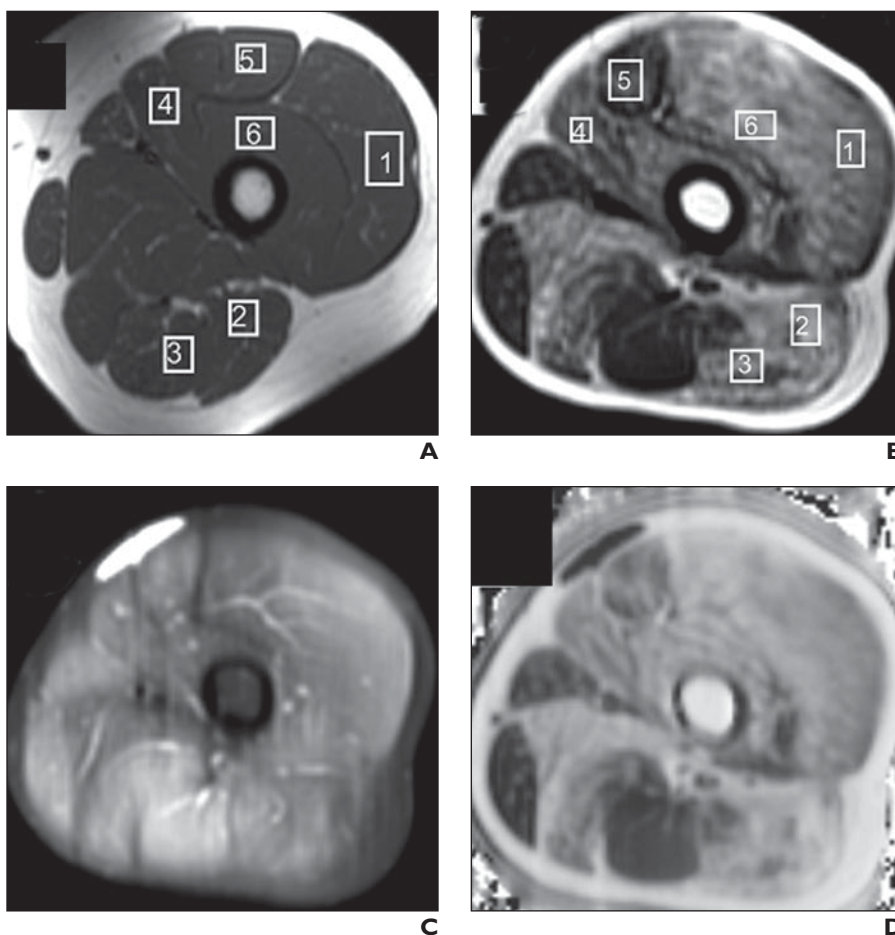
### Functional and Strength Evaluations

Subjects were examined by a pediatric neurologist, and a grade of functional ability based on the scale proposed by Brooke et al. [9] (Table 1) was determined. Manual muscle testing of the iliopsoas, hip adductor, hip abductor, quadriceps, ankle dorsiflexor, and ankle plantar flexor muscles was performed according to Medical Research Council (MRC) guidelines [14]. On the basis of a grade of 0–5 for each muscle, a lower extremity strength score was calculated as  $MRC\ percentage = (\text{sum of grade scores} \times 100) / (\text{number of muscles tested} \times 5)$ . Strength of the knee extensor muscles was measured with an isokinetic dynamometer (Kin-Com 125AP, Chattanooga Group). The subject was positioned on the dynamometer seat with the distal shin secured against a pad attached to the lever arm of the dynamometer and the knee joint axis aligned with the center of rotation of

the lever arm. The dynamometer moved the knee from full flexion to full extension at 20%/s, and the subject was asked to actively assist the motion (push against the pad as hard as possible to make the machine move faster). Knee extension moment was measured and corrected for gravity. The maximum moment from several trials normalized according to body mass was used for analysis.

### MRI Measurements

MR images were acquired on a 1.5-T system (LX CVi, GE Healthcare) with state-of-the-art imaging, including standard T1-weighted spin-echo and Dixon sequences. The total amount of time required for the MRI examination was approximately 10 minutes. No sedation or contrast material was used. Care was taken to position the subjects and to adjust the number of slices so that the same anatomic extent of the muscles of interest



**Fig. 1**—10-year-old boy with Duchenne muscular dystrophy and 9-year-old healthy boy. **A** and **B**, T1-weighted spin-echo MR images (TR/TE, 400/10) show regions of interest on previously obtained comparison image of 9-year-old healthy boy (**A**) and in 10-year-old patient with functional score of 3 (**B**). 1 = vastus lateralis, 2 = biceps femoris, 3 = semitendinosus, 4 = vastus medialis, 5 = rectus femoris, 6 = vastus intermedius. **C**, MR image shows results of Dixon water measurement in patient. **D**, MR image shows computed percentage fat in patient.

**TABLE 1: Functional Grades According to Scale of Brooke and Associates**

Grade	Function
1	Walks and climbs stairs without assistance
2	Walks and climbs stairs with aid of railing
3	Walks and climbs stairs slowly with aid of railing (12 s for four standard stairs)
4	Walks unassisted and rises from chair but cannot climb stairs
5	Walks unassisted but cannot rise from chair or climb stairs
6	Walks only with assistance or walks independently with long leg braces
7	Walks in long leg braces but needs assistance for balance
8	Stands in long leg braces but unable to walk even with assistance

Note—Adapted from [9].

was scanned for each subject. The amount of fat in muscle was determined with the three-point Dixon technique, which allows separation of MR signal intensity into the individual contributions of fat and water in each voxel of tissue [19]. Fat and water images were loaded into a custom-made program (IDL, Interactive Data Language), and quantitative fat images (percentage fat) were computed as follows:  $\text{percentage fat} = \frac{SI_{\text{fat}}}{SI_{\text{fat}} + SI_{\text{water}}}$ , where  $SI_{\text{fat}}$  is signal intensity in the fat image and  $SI_{\text{water}}$  is the signal intensity from the water image in each pixel. These images were used to quantify the lipid content in regions of interest defined in six muscles: rectus femoris, vastus lateralis, vastus intermedius, vastus medialis, biceps femoris, and semitendinosus (Fig. 1). In vivo reproducibility for three-point Dixon measurements of muscle fat fraction in healthy children has been previously calculated to be 2.3% [21]. Three investigators reviewed all Dixon images. Of the images that displayed all muscle groups of interest, the center image was selected for further analysis. Regions of interest were then placed on different muscle groups. Consensus among the investigators was reached in each case.

**Statistical Analysis**

Correlations were performed to examine the association between the various measures of disease progression. Pearson's correlation was used for continuous variables, and Spearman's rank correlation was used for ordinal variables.

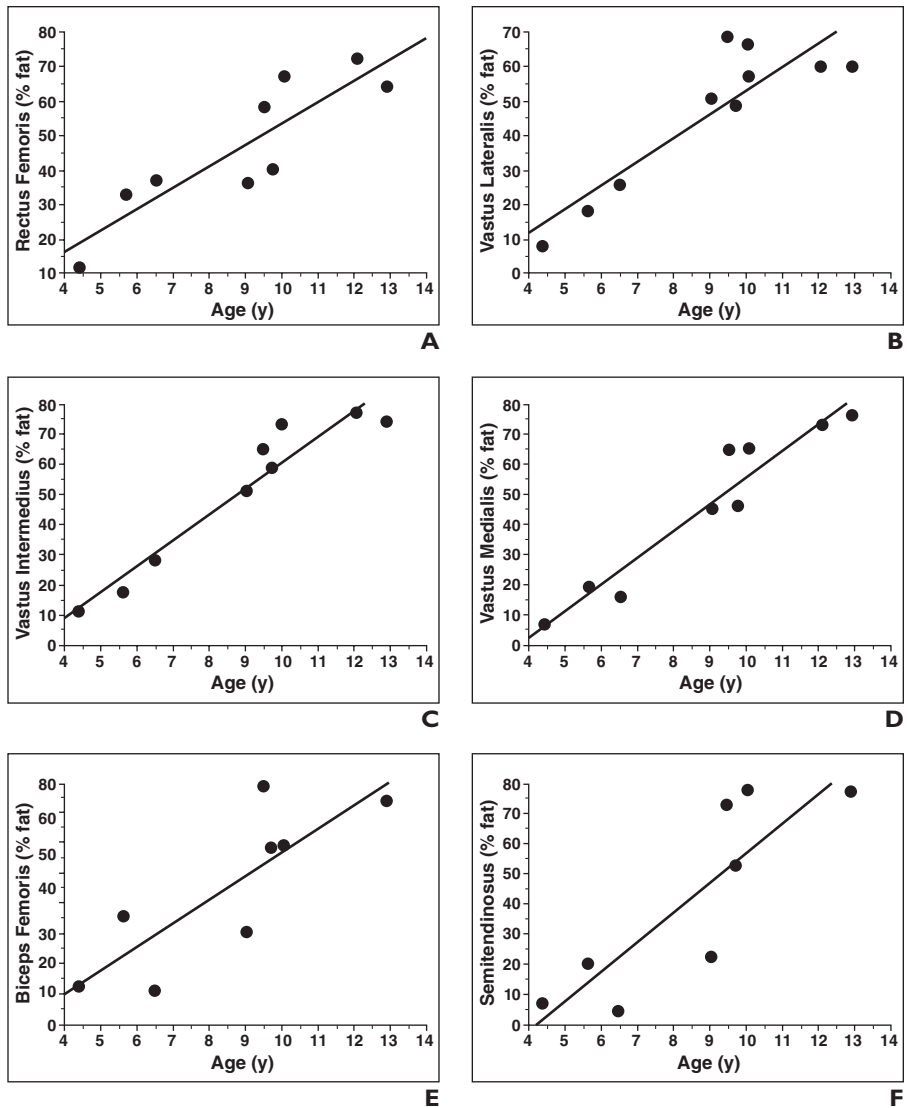
**Results**

Strong correlations were found between three-point Dixon measurements of fat percentage among all the muscles examined in this study. Correlations were  $\geq 0.88$  for the muscles within the quadriceps and hamstring muscle groups and  $\geq 0.83$  between the two muscle groups (Table 2). Regardless of the muscle examined, fat percentage increased with age in subjects with DMD ( $r^2 = 0.68\text{--}0.92$ ;  $p < 0.02$ ) (Fig. 2). In contrast, there were insignificant or no correlations between age and strength calculated with MRC percentage ( $r^2 = 0.22$ ;  $p = 0.20$ ) and dynamometer measurements of knee extensor torque ( $r^2 = 0.00$ ;  $p = 0.98$ ).

Functional grade was strongly inversely correlated with fat percentage measured with the Dixon technique ( $p < 0.05$ ) (Fig. 3). Correlations between worsening functional grade and strength measured as MRC percentage were not statistically significant ( $p = 0.07$ ) and were nonexistent for dynamometer measurements of knee extensor torque ( $p = 0.54$ ). The average fat percentage of the six

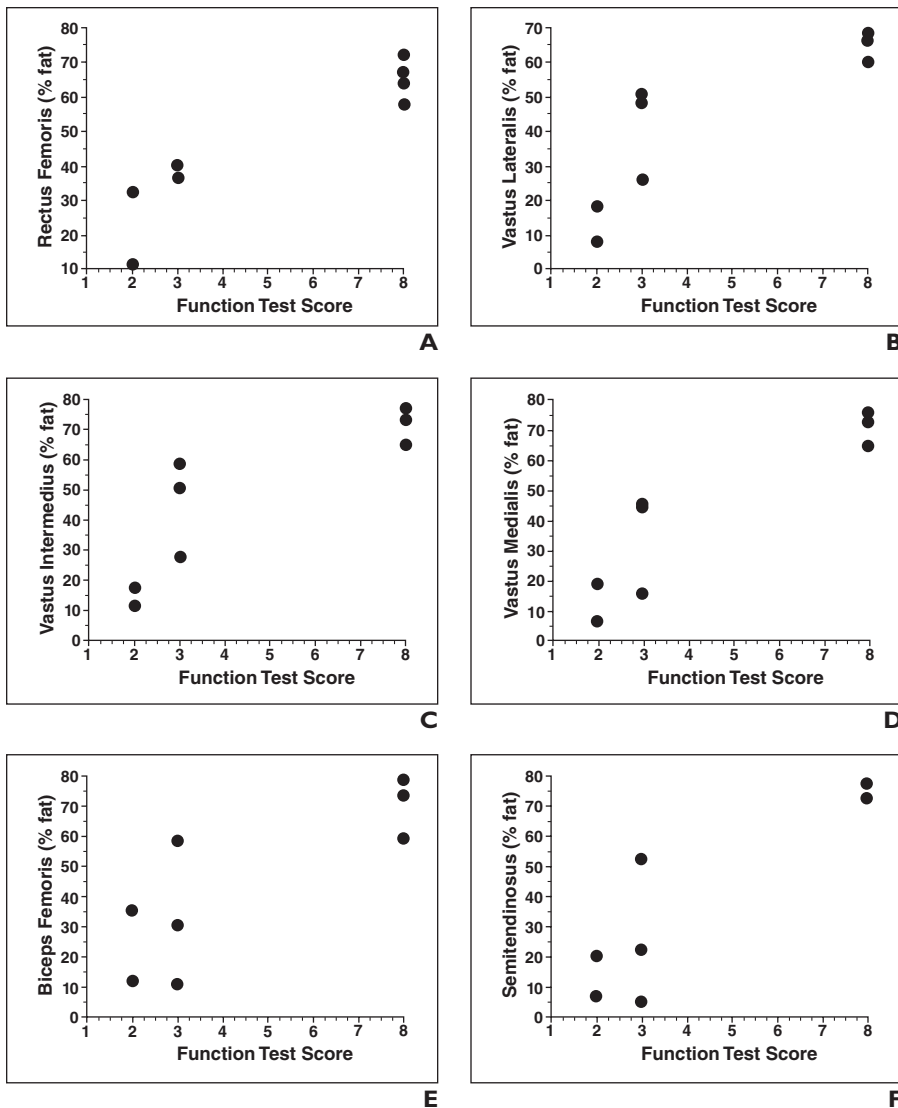
**TABLE 2: Percentage Fat Correlations on Dixon MRI**

Muscle	Rectus Femoris	Vastus Lateralis	Vastus Intermedius	Vastus Medialis	Biceps Femoris	Semitendinosus
Rectus femoris	1.00	0.88	0.91	0.93	0.83	0.90
Vastus lateralis		1.00	0.97	0.95	0.85	0.89
Vastus intermedius			1.00	0.98	0.86	0.91
Vastus medialis				1.00	0.91	0.94
Biceps femoris					1.00	0.96
Semitendinosus						1.00



**Fig. 2**—Graphs show Dixon MRI measurements of intramuscular fat percentage increased significantly with age in all muscles examined. Data points for biceps and semitendinosus for one patient were omitted because of poor magnetic field homogeneity and compromised separation of lipid and water signals.  
**A**, Rectus femoris muscle ( $y = -8.6 + 6.2 * x$ ;  $r^2 = 0.78$ ;  $p = 0.002$ ).  
**B**, Vastus lateralis muscle ( $y = -16.3 + 6.9 * x$ ;  $r^2 = 0.78$ ;  $p = 0.002$ ).  
**C**, Vastus intermedius muscle ( $y = -25.5 + 8.6 * x$ ;  $r^2 = 0.92$ ;  $p < 0.0001$ ).  
**D**, Vastus medialis muscle ( $y = -33.0 + 8.8 * x$ ;  $r^2 = 0.92$ ;  $p < 0.0001$ ).  
**E**, Biceps femoris muscle ( $y = -22.0 + 7.9 * x$ ;  $r^2 = 0.68$ ;  $p = 0.01$ ).  
**F**, Semitendinosus muscle ( $y = -40.9 + 9.8 * x$ ;  $r^2 = 0.73$ ;  $p = 0.007$ ).

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**Fig. 3**—Graphs show Dixon MRI measurements of intramuscular fat percentage increased significantly with decreasing functional level in all muscles examined. **A–F**, Graphs show rectus femoris (**A**), vastus lateralis (**B**), vastus intermedius (**C**), vastus medialis (**D**), biceps femoris (**E**), and semitendinosus (**F**) muscles.

muscles was not related to strength measured as MRC percentage ( $r^2 = 0.34$ ;  $p = 0.12$ ) or with the dynamometer ( $r^2 = 0.01$ ;  $p = 0.82$ ).

### Discussion

The histopathologic characteristics of DMD are hallmarked by variations in fiber size, myofiber degeneration, infiltration of inflammatory cells, and formation of foci of fibrosis and fatty infiltration [22]. The purpose of this study was to assess these histopathologic features with imaging measurements of fatty infiltration obtained with advanced MRI techniques. We found that quantitative three-point Dixon values of muscle adiposity

accurately reflect disease severity in patients with DMD. These measures of fat infiltration correlated more strongly with disease progression indicated by functional grade than did results of manual muscle testing and measurements of muscle strength obtained with a dynamometer. Therefore, the Dixon MRI technique may greatly facilitate multicenter trials involving patients with DMD.

A variety of imaging techniques have been studied as possible adjuncts to physical examination or as means of further exploration of the pathophysiologic mechanism of DMD. Both CT and sonography have been used to detect variations in muscle volume and the

degree of fatty infiltration in boys with DMD and other neuromuscular disorders [23–28]. Developments in MRI techniques have prompted investigators to explore the advantages of better spatial and contrast resolution [12]. MRI has been used to show the pattern of age-related changes in muscle bulk and fatty infiltration in the lower extremities of untreated boys [11]. Other small studies in which MRI was used showed abnormalities in muscle size [16] and structure [17, 18] in patients with DMD. An elegant imaging sequence for discrimination between fat and water spins on the basis of their resonant frequency difference was introduced by Dixon in 1984 [19]. This technique, in part developed to overcome sensitivity to magnetic field inhomogeneity, has been found highly reproducible, accurate, and useful for in vivo quantification of fat in lean tissues, such as skeletal muscle [20–21].

There were several limitations to our study. The number of patients examined was small, and although we included all grades of disease severity, most of the boys were in the early or late stages of disease. However, even after allowance for these limitations, the potential of the three-point Dixon technique compared with standard assessments of muscle function was striking. In addition, by design we tried to avoid the large variations in muscle damage known to occur in DMD patients by examining only muscles most commonly affected [11]. This selectivity may have accounted for the strong correlations between three-point Dixon measurements of fat percentage among muscles. Nevertheless, MRI enables comprehensive evaluation of muscle damage in DMD patients, including detection of subtle and subclinical changes in individual muscles that cannot be isolated from their anterior or posterior muscle groups with strength testing.

Clinical trials involving patients with DMD are hindered by difficulties in finding suitable objective end points. Although simple, safe, and inexpensive, manual muscle tests depend on patient effort and are limited by intraobserver and interobserver variability. Measurement of muscle force with a dynamometer is more quantitative but has limitations in terms of effort and examiner (test setup) variability. Functional testing, such as time to walk a set distance, is effort dependent, and the results change with fatigue throughout the day [14]. In contrast, three-point Dixon measurement of adipose tissue in muscle is objective and highly reproducible because it is not influenced by fatigue

[21]. Therefore, this technique may be better than conventional clinical functional grading systems in prediction of progression of disease and therapeutic response.

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