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Relationships of thigh muscle contractile and non-contractile tissue with function, strength, and age in boys with Duchenne muscular dystrophy

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Abstract

The purpose of this study was to assess the contractile and non-contractile content in thigh muscles of patients with Duchenne muscular dystrophy (DMD) and determine the relationship with functional abilities. Magnetic resonance images of the thigh were acquired in 28 boys with DMD and 10 unaffected boys. Muscle strength, timed functional tests, and the Brookes Lower Extremity scale were also assessed. Non-contractile content in the DMD group was significantly greater than in the control group for six muscles, including rectus femoris, biceps femoris-long head and adductor magnus. Non-contractile content in the total thigh musculature assessed by MRI correlated with the Brookes scale ($r_s=0.75$) and supine-up test ($r_s=0.68$), as well as other functional measures. An age-related specific torque increase was observed in the control group ($r_s=0.96$), but not the DMD ($r_s=0.06$). These findings demonstrate that MRI measures of contractile and non-contractile content can provide important information about disease progression in DMD.

Keywords

Duchenne muscular dystrophy; muscle strength; magnetic resonance imaging

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1. Introduction

Duchenne muscular dystrophy (DMD) is one of the most common inherited and degenerative diseases of skeletal muscle with an incidence of 1:3500 male births. DMD is caused by a genetic defect on the short arm of the X-chromosome at Xp21, which results in the absence of the cytoskeletal protein dystrophin [1]. The disease is characterized by progressive weakness of skeletal muscle leading to loss of ambulation between the age of 10 and 15 years and premature death due to cardiopulmonary complications in the late teens or twenties [2, 3].

Skeletal muscles in boys with DMD display important structural and compositional changes, with progressive fatty tissue infiltration and muscle atrophy, secondary to structural weakness of the sarcolemma [4–7]. Pathophysiological abnormalities and muscle weakness are first apparent in the large proximal hip and shoulder muscles, and later spread to the distal extremities and the trunk. In the lower extremity muscles, disease progression appears to be more rapid in the gluteus maximus and adductor magnus, followed by the quadriceps femoris and biceps femoris. In contrast, there is selective sparing of specific hamstrings and adductor muscles [5, 7, 8].

The goal of most therapeutic interventions for muscular dystrophy is restoration of the dystrophin/dystrophin-associated glycoprotein complex and maintenance of ambulatory function. Preservation of independent walking not only has an important impact on quality of life, it also delays the development of scoliosis, and ultimately may prolong both physical functioning and lifespan in boys with DMD. Since the thigh muscles play an important role in standing and locomotion, a careful assessment of their pathoanatomical alterations during disease progression is warranted. Surprisingly, few studies [4, 9] have quantified the loss of contractile tissue and accumulation of non-contractile content in individual thigh muscles of boys with DMD and assessed its effect on force production and/or functional ability.

Magnetic resonance imaging (MRI) is a powerful non-invasive tool that has been used to monitor disease progression in boys with DMD [7–11]. Several MRI methods, such as T₁- and T₂-weighted imaging, short-tau inversion recovery imaging, and three-point Dixon imaging, have been used to estimate intramuscular fatty infiltration in DMD patients [7–9, 11]. In addition, MRI has been used extensively to quantify alterations in muscle contractile cross-sectional area (CSA) during disuse atrophy and aging [12–17]. Recently, using T1-weighted imaging, we demonstrated differences in age-related changes in lower extremity muscle CSA and specific torque production in ambulatory boys with DMD compared to controls [10]. Assessment of the non-contractile versus contractile fractions of muscle tissue may provide additional information on the relationship between muscle size and function in boys with DMD. To date, few studies have performed quantitative evaluations of the contractile and non-contractile CSA in individual thigh muscles of boys with DMD and assessed changes in specific torque (torque per unit of contractile area) production with age.

Therefore, the purposes of this study were: 1) to assess the non-contractile content and contractile CSA in individual muscles of the thigh in boys with DMD and age-matched healthy controls, 2) to evaluate the relationship between the proportion of non-contractile content in thigh muscles, assessed by MRI, and their functional ability, and 3) to assess the knee extensor specific torque production in boys with DMD versus controls, and determine its relationship with age.

2. Materials and Methods

2.1 Subjects

A cohort of twenty-eight boys with DMD (DMD group) and ten healthy boys from the general population (CONT group) volunteered to participate in this observational cross-sectional study. Thirteen of the DMD subjects participated in a previous MR study performed at our site [10]. Ambulatory status and mean ($\pm$ SEM) physical characteristics of all subjects are shown in Table 1. A report confirming the diagnosis of DMD using molecular genetic testing (e.g. PCR amplification) and/or immunohistochemical staining from muscle biopsy was obtained from each of the DMD subjects. In the DMD group, twenty-four boys were ambulatory and four boys were non-ambulatory. All DMD subjects were being treated with corticosteroids (either Prednisone or Deflazacort). CONT subjects were relatively sedentary, in that they did not participate in sport specific training 2 or more times per week. This study was approved by the Institutional Review Board at the University of Florida. Written informed consent was obtained from the participants' parents, and written assent was obtained from each participant.

2.2 MRI Acquisition

MRI was performed on either a 1.5T (Signa, GE Medical Systems) or 3.0T (Achieva, Philips Medical Systems) whole-body scanner. Subjects were placed in a supine position and images of the thigh were acquired using a flexible receive only surface coil. T₁-weighted 3-D gradient-echo transaxial images of the right-thigh were collected with the following parameters (for 1.5T or 3.0T): repetition time=18 ms or 24 ms, echo time=3.4 ms or 1.8 ms, flip angle=10° or 20°, voxel resolution=0.75 mm, optimized field of view=14 × 14 cm, slice thickness=7 mm or 2.8 mm.

2.3 MRI Data Analysis

Medical Image Processing, Analysis and Visualization (MIPAV) software (version 4.2.1; National Institutes of Health, MD) was used to analyze the MR images. We selected a single image that corresponded to the most proximal slice in which the biceps femoris-short head (BFs) could be visually confirmed. There were three reasons we chose this image: 1) all targeted muscles were identified at this level of the image, 2) the position was in the mid-thigh region along the length of the thigh [12], and 3) the image corresponded to the maximum CSA of the quadriceps femoris (QF) [12]. We identified twelve individual muscles on the image: rectus femoris (RF), vastus lateralis (VL), vastus intermedius (VI), vastus medialis (VM), BFs, biceps femoris-long head (BFL), semitendinosus (ST), semimembranosus (SM), adductor longus (AL), adductor magnus (AM), gracilis (Gr) and sartorius (Sar). Representative MR images for DMD and CONT subjects are shown in Fig. 1. QF (i.e. sum of RF, VL, VI and VM), hamstrings (HM) (i.e. sum of BFs, BFL, ST and SM), and hip adductors (AD) (i.e. sum of AL, AM, Gr and Sar), were also evaluated as muscle groups. Contiguous axial images were used to help identify each of the muscles and in particular the muscle boundaries.

We calculated the contractile and non-contractile CSA in the muscles using a modified image analysis technique as previously described [14, 16, 17]. The first step of the analysis was to correct for image heterogeneity caused by suboptimal radiofrequency coil uniformity, or gradient-driven eddy currents, using a well-established nonparametric nonuniform intensity normalization (N3) algorithm [18]. This step was essential for subsequent analyses that assume homogenous signal intensity across the images. Optimized image correction parameters (N3) were determined (end tolerance 0.0001; maximum iterations 100, signal threshold 1; field distance 33.33 mm; subsampling factor 2; Kernal full width half maximum of 0.15; Wiener filter noise 0.01), and the same parameters were applied to all images. The

investigator then drew a region of interest (ROI) containing ~50% muscle and 50% subcutaneous fat by manually tracing the Sar and Gr (Fig. 2-A & B). These muscles were chosen as a reference as they have selective sparing in subjects with DMD [5, 7]. The total number of pixels within the two ROIs and a frequency distribution and histogram of all pixels and signal intensities were produced. Representative histograms for DMD and CONT groups are presented in Fig. 2-C & D. To separate contractile and non-contractile tissues in the pixel number-signal intensity histogram with minimal investigator bias, we implemented the Maximum Entropy method, a reliable histogram shape-based thresholding technique used in medical imaging analysis [19] (Fig. 2-C & D). To minimize manual tracing-induced error on thresholding values, five trials were averaged, and the values were applied to all muscles of interest. After carefully tracing the edge of each muscle as shown in Fig. 2-A & B, the following parameters were calculated: 1) the total number of pixels within the ROI; 2) the number of pixels with a signal intensity lower than the threshold value (contractile tissue), and 3) the number of pixels with a value higher than the threshold value (non-contractile tissue). The proportion of contractile and non-contractile content of each muscle was then calculated as follows:

$$\begin{aligned}\text{Contractile content (\%)} &= (\text{CC}) / [(\text{CC}) + (\text{non} - \text{CC})] \times 100 \\ \text{Non} - \text{contractile content (\%)} &= (\text{non} - \text{CC}) / [(\text{CC}) + (\text{non} - \text{CC})] \times 100,\end{aligned}$$

where CC = contractile tissue pixel number; non-CC = non-contractile tissue pixel number

Subsequently, the contractile and non-contractile CSA was calculated by the following equations:

$$\begin{aligned}\text{Contractile CSA (cm}^2\text{)} &= (\text{CC}) * (\text{FOV} / \text{matrix size})^2 \\ \text{Non} - \text{contractile CSA (cm}^2\text{)} &= (\text{non} - \text{CC}) * (\text{FOV} / \text{matrix size})^2\end{aligned}$$

where CC = contractile tissue pixel number; FOV = field of view; non-CC = non-contractile tissue pixel number

All images were read in random order, and one investigator (HA) performed all of the analyses. Test-retest reliability of non-contractile content, contractile CSA and non-contractile CSA in 10 DMD subjects was assessed. Intraclass correlation coefficients (ICC (2, 1)) in individual muscles, except for BF, showed values ranging between 0.97 to 1.00 for non-contractile content, 0.98 to 1.00 for contractile CSA and 0.96 to 1.00 for non-contractile CSA (all $p < 0.001$). The ICCs of BF were 0.81 ($p = 0.05$), 0.99 ($p < 0.001$) and 0.87 ($p < 0.01$) for non-contractile content, contractile CSA and non-contractile CSA, respectively.

2.4 Isometric Knee Extension Peak Torque

Isometric knee extension peak torque of the right leg was measured using an isokinetic dynamometer (Biodex System 3 Pro, Biodex Medical Systems, Inc.). The hip and upper body were secured with straps to the dynamometer, and the hip and knee joint angles were set at 90°. The subject was instructed to push as hard as possible for 5 seconds with 2 minutes of rest between trials. Five trials were performed, and the highest torque value, i.e. peak torque, was used for further analysis. We tested for between-day reliability with two testing sessions 2 months apart in children with DMD ($n = 6$) and controls ($n = 10$). ICCs (2, 1) were calculated for the subjects, and high reliability was found (ICC = 0.89 in DMD and 0.99 in controls, $p < 0.001$ for both ICCs) [10].

Isometric knee extension peak torque was expressed both as an absolute value (Nm) and as the ratio of peak torque to contractile CSA of the QF, i.e. specific torque (Nm/cm²). Specific torque was used as an index of the force generation capacity of the QF [20].

2.5 Functional Abilities

Subjects performed five functional tasks that were timed using a stopwatch. These tasks included the time to walk 30 feet (30ft Walk), rise from the floor (Supine Up), rise from a chair (Chair Up), the pediatric timed up and go (TUG) and ascend four stairs (4 Stairs) [6]. Subjects performed each task three times, and the fastest time was recorded for analysis. The functional ability of the DMD subjects was also ranked using the Brookes Lower Extremity Functional Scale [21]. This scale ranged from grade 1 (able to walk and climb stairs independently) to grade 10 (confined to bed).

2.6 Statistical Analysis

All values are reported as means and standard error of the mean. Levene's test showed unequal variance between DMD and CONT groups. Accordingly, non-parametric Mann-Whitney tests were used to compare non-contractile content, contractile CSA and non-contractile CSA between DMD and CONT groups. Kruskal Wallis tests were used to analyze non-contractile content among age categorized groups or muscles within the same age category. Spearman's rank correlation tests were used to determine the relationships between two independent variables. SPSS statistical package (Version 17.0J for Windows, SPSS Inc, Chicago, IL) was used for all analysis. The level of significance was set at $p < 0.05$; however the level of significance was adjusted by Bonferroni correction when appropriate.

3. Results

3.1 Subject demographics

The demographics for the DMD and CONT groups are shown in Table 1. Boys with DMD were shorter in height; however no significant differences were noted for age, weight or BMI between the two groups. For the DMD group, the mean Brookes scale was 2.8 ± 0.5 ranging from grade 1 to 9. This scale indicates that the majority of patients in this group were ambulating with minimal to moderate difficulty, except for four subjects who were non-ambulatory (grade 9).

3.2 Contractile and non-contractile contents of thigh muscle groups and specific thigh muscles

Contractile CSA (cm²), non-contractile CSA (cm²) and non-contractile content (%) of individual muscles for the DMD and CONT groups are shown in Table 2. Significant loss of contractile CSA was noted in specific dystrophic muscles, including the VM ($p < 0.001$), BF ($p < 0.01$) and AM ($p < 0.001$) muscles. The non-contractile CSA of the RF ($p < 0.01$), BFL ($p < 0.05$), ST ($p < 0.05$) and AM ($p < 0.01$) in the DMD group were significantly higher compared to the CONT group. In addition, the proportion of non-contractile content in the RF ($p < 0.01$), VI ($p < 0.05$), VM ($p < 0.05$), BFL ($p < 0.05$), SM ($p < 0.05$) and AM ($p < 0.01$) was significantly higher in the DMD compared to the CONT group.

The contractile CSA and non-contractile content for the three muscles groups QF, HM, and AD as well as the total thigh (sum of QF, HM, AD) in both subject groups are shown in Fig. 3. The contractile CSA in the QF ($p < 0.01$), AD ($p < 0.001$) and Total thigh ($p < 0.01$) for the DMD group was significantly lower than those in the CONT group corresponding to 63%, 64% and 69% of CONT values, respectively; however no significant difference was observed in the HM (DMD: 91% of CONT group). In addition, the proportion of non-

contractile content in the QF ($p < 0.01$), HM ($p < 0.05$), AD ($p < 0.001$) and Total thigh ($p < 0.01$) for the DMD group was significantly greater than the CONT group.

Four of the boys in the DMD group were non-ambulatory. Non-contractile content in the individual thigh muscles for these four subjects showed considerable variability, with values ranging from 10% (AL) to 84% (BFI), 4% (Sar) to 93% (BFI), 4% (AL) to 93% (BFI), and 17% (Gr) to 100% (AM), respectively. In all four subjects the BFI muscle showed $>80\%$ (84–93%) non-contractile content, while Gr showed only an average of 11% (6–17%).

3.3 Age-related non-contractile content in specific thigh muscles

Four muscles were selected for evaluation of age-related changes in non-contractile content. In specific we selected one muscle from each muscle group that showed the greatest proportion of non-contractile content as well as the more preserved Gr muscle. The non-contractile content for the BFI, RF, AM and the Gr in different age groups are shown in Fig. 4. Starting from the age group 8–10 years, progressive increases in the proportion of non-contractile tissue were noted. The non-contractile content in the BFI, RF and AM for both the 10 to <12 years age group and ≥ 12 years age group was significantly higher than that in the <8 years age group (all $p < 0.02$). As expected the Gr muscle showed a distinctly more preserved pattern, even in the oldest age group (≥ 12 years).

3.4 Relationships between non-contractile content in thigh muscle groups and functional abilities

Spearman's rank correlation coefficients were used to examine the relationships between the non-contractile content in muscle groups, timed functional tests and the Brookes scale in DMD subjects (Table 3 and Fig. 5). Significant relationships were noted between almost all tested pairs of independent variables. Among timed functional tests, Supine Up resulted in higher correlation coefficients than the other functional tests for all muscle groups of the thigh (Fig. 5). Significant correlation coefficients were also found between non-contractile content in all tested pairs of muscle groups and the Brookes scale ($r_s = 0.71$ to 0.77 , all $p < 0.01$).

3.5 Isometric peak torque, specific torque and age in DMD

The relationships between peak torque during isometric knee extension and contractile CSA of the QF and between specific torque and age are shown in Fig. 6. There was a significant correlation between peak torque and contractile CSA of the QF for both the DMD and CONT groups (DMD, $r_s = 0.78$, $p < 0.01$; CONT, $r_s = 0.90$, $p < 0.01$). However, there was an evident shift in the slope, indicating a dramatic reduction in the specific torque production of boys with DMD (0.9 ± 0.1 Nm/cm² in DMD vs 1.8 ± 0.2 Nm/cm² in CONT, $p < 0.001$). Interestingly, there was no significant correlation between specific torque and age for the DMD group ($r_s = 0.06$) with specific torque values remaining relatively constant with age; whereas there was a strong relationship in the CONT group ($r_s = 0.96$, $p < 0.01$).

4. Discussion

The main findings of this study were that: 1) boys with DMD had a significant greater proportion of non-contractile content in all three thigh muscles groups compared to the CONT group, with particularly high values in the RF, BFI, and AM muscles; 2) the latter muscles showed a significant increase in non-contractile content starting at the age group 10–12 years; 3) the proportion of non-contractile content in the total thigh muscles, as well as individual muscle groups, correlated with functional ability as assessed by the Brookes scale, Supine Up, TUG, Chair Up, and 4 stairs; and 4) specific knee extensor torque increased with age in the CONT group but not in boys with DMD. Collectively, these results

demonstrate that noninvasive MRI measures of contractile and non-contractile tissue in the thigh muscles of boys provide valuable information on disease progression in boys with DMD and are related to the loss in functional ability.

4.1 Assessment of non-contractile content in DMD thigh muscles

We found that the proportion of non-contractile content in six individual thigh muscles (RF, VI, VM, BFL, SM and AM) was significantly greater in the DMD group compared to CONT group (Table 2). The most sparing was observed in the Gr, AL and Sar muscles with average non-contractile content values below 10% in all three muscles. We also found that the non-contractile content in the RF, BFL and AM was significantly greater in the two older age groups (10 to <12 years and ≥ 12 years) compared to the non-contractile content measured in the <8 years age group (Fig. 4). Using quantitative computer tomography scanning, Liu et al. [5] showed that the rate of muscle fiber loss and fatty tissue replacement was fastest in the BF, AD and QF followed by the ST and SM; with the Sar and Gr remaining relatively unaffected until later stages in 71 DMD subjects. Our results generally support the findings from Liu et al. and confirm that there is considerable variation in the rate of disease progression across thigh muscles, and even within muscle groups.

Another noteworthy finding from this study is that there is considerable variation in the non-contractile content in individual muscles within the same functional muscle groups. This was particularly prevalent in the hip adductor muscles, which contained the three most preserved muscles (Sar, Gr, and AL demonstrating only 3–8% of non-contractile tissue) as well as one of the most severely affected muscles (AM with 30% non-contractile content). This result is supported by findings from previous studies qualitative MRI and CT studies that reported varying non-contractile content patterns with disease progression among synergistic muscles [4, 5].

4.2 Relationship between non-contractile tissue content and functional abilities

We found significant relationships between the non-contractile content of each of the three muscle groups as well as total thigh and timed functional tests (Table 3 and Fig. 5). Wren et al. [9] used three-point Dixon MRI and reported that fat percentage in six muscles of the thigh (i.e. RF, VL, VI, VM, BF and ST) correlated strongly with functional test scores in all the muscles examined. Liu et al. [4] also showed a significant correlation between clinical functional grading ($r_s = 0.88$, $p < 0.01$) and the qualitative MR grading score in 29 boys with DMD. Our results confirm the findings of these previous studies and showed the highest correlation between non-contractile content in muscle groups and performance on the Supine Up test as well as the Brooks scale (Table 3 and Fig. 5). Interestingly, the variance of the Supine Up performance in boys with DMD was statistically explained by the proportion of non-contractile tissue in the thigh muscles ($r_s = 0.56$ – 0.79), without taking into account the contribution from the trunk and arm muscles. This finding suggests that fatty infiltration in the thigh muscles may proceed at a similar speed to the other working muscles involved in performing Supine Up [6]. The Brooks scale showed a strong correlation with the non-contractile content in each of the muscle groups with r_s -values ranging between 0.71 and 0.77 (Table 3).

4.3 Muscle contractile CSA and knee extensor torque production

Contractile CSA is a valuable measurement in disease conditions, since muscle size is the major determinant of muscle torque [22] and a decrease in activity level secondary to the disease is expected to contribute to loss of muscle mass. It is noteworthy that the contractile CSA of the QF and AD in the DMD group was significantly smaller (63% and 64% in QF and AD, respectively) than that of the CONT group, while there was no difference in HM contractile CSA (DMD, 91% of CONT).

As expected peak torque was strongly correlated with contractile CSA of the QF for both the DMD and CONT groups. However, as shown in Fig. 6-A, the distribution of the scatter plot was dramatically different in the DMD group. More interestingly, the relationship between age and specific torque was distinctly different in the DMD group compared to the CONT group (Fig. 6-B). While specific torque was strongly correlated with age in the CONT group this relationship was absent in the DMD group, suggesting that DMD boys do not demonstrate an increase in torque generating capacity during growth and development. Similar results were reported by Skalsky et al. [23] who showed that peak isometric knee extension strength per regional lean mass, assessed using DEXA, was significantly lower in children with DMD than in controls. Although specific torque might not be as accurate as specific tension as a measure of the force-generating capacity of muscle [24, 25], the distinct differences observed in age-related specific torque between the two groups are indicative of underlying physiological differences in skeletal muscle. Possible explanations for the differences may include impaired motor unit recruitment during force exertion and impaired tension development at the level of individual fibers. A significant loss in specific tension of skinned dystrophic fibers has been reported in *mdx* mice [26, 27], as well as the Golden retriever MD dog [28] and patients with myotonic dystrophy [29]. The loss of specific tension, P_0/CSA , in skinned dystrophic fibers, absent of the sarcolemmal membrane, has been postulated to be related to a decrement in myofibrillar protein content, secondary to the imbalance in protein synthesis and degradation in dystrophic muscle [30].

Somewhat surprisingly, we did not observe a progressive age-dependent decrement in knee extensor specific torque in the DMD population. In fact, specific torque remained relatively constant in the developing boys with DMD, whereas a 3-fold increase in specific torque production was observed in healthy boys between the ages of 8 and 15. An increase in muscle performance per unit muscle CSA during childhood and adolescence has previously been reported for both the lower leg [20, 31] and arm [32] muscles. The physiological basis for the large gains in muscle performance in healthy boys is not well established, although improved voluntary activation and especially frequency of motor unit recruitment [32] and a shift in fiber type composition [33] have been implicated. Conversely the absence of age dependent gains in muscle performance per unit contractile CSA in boys with DMD may reflect a lack of maturation of the neuro-muscular system. Several recent studies have argued that dystrophin has a direct role in promoting the maturation of synaptic folds and is important for the maturation of the neurotransmitter receptor complexes as well as the regulation of neurotransmitter release at the NMJ synapses [34, 35].

4.4 Methodological considerations

In this study, we quantified the contractile and non-contractile content in individual thigh muscles in boys with DMD using MR methodology previously described for assessments in elderly healthy subjects, women and individuals with spinal cord injury [14–17]. Overall our results in the CONT groups were consistent with the literature with $1.7 \pm 1.5\%$ of mean non-contractile content in individual muscles, ranging from 0.7% in the VI to 3.5% in the Sar. Elder et al. [14] and Gorgey et al. [15] reported 2% and 5% non-contractile content in the thigh of healthy young men and women, respectively. Therefore, our results in CONT subjects appear to be similar to those of previous studies, especially considering the differences in the subjects' physical characteristics and muscles tested. Interestingly, these values are somewhat lower than values reported for fibro-fatty infiltration in healthy human subjects based on muscle biopsies. Using conventional stereological (point counting) techniques normal upper limits of fat-fibrosis in biopsies of the QF are reported to be around 12% [36]. In comparing imaging and muscle histology results, it is important to keep in mind that imaging methods such as MR and CT are limited in spatial resolution and suffer from partial volume filling which could be occupied by collagen or fat cells.

There are some other methodological limitations to consider in this study. First, we could not completely correct for image inhomogeneity, even using N3 shading correction with optimized parameters (see Fig. 1). However, we did not find any systematic errors that may be caused by incomplete shading, thus we speculate that the effect is negligible. Also, we can not exclude the possibility that an error resulted in overestimation of the specific torque production in one boy in the DMD group, as the reported specific torque for this subject was several fold higher than other subjects around the same age (see Fig. 6). Given that this subject had the highest amount of fatty tissue infiltration, it is possible that the contractile CSA in the QF of this subject may be underestimated (5.1 cm²). Partial volume filling in severely affected muscles leads to broadening of the histogram components and likely underestimation of the remaining muscle fibers; since the majority of pixels contain some component of fat.

In conclusion, in this study we used a straightforward T1 magnetic resonance imaging strategy, which can easily be applied in clinical magnets, to quantify the contractile versus non-contractile tissue content in individual thigh muscles of boys with DMD and determined its relationship with functional abilities as well as strength. The proportion of non-contractile content, assessed by MRI, in the DMD group was significantly greater than that of the CONT group for the RF, VI, VM, BFL, ST and AM. Also, significant correlations were observed between the non-contractile content in the QF, HM, AD and Total thigh and the Brookes scale, TUG, Supine Up, Chair Up and 4 stairs. The highest correlations were observed with Supine Up and the Brooks scale. Muscle atrophy, defined as a loss in contractile CSA, was observed in the QF and AD muscle groups, but not in the HM. Importantly, the relationship between knee extensor torque production and contractile CSA, was dramatically different in boys with DMD compared to controls, consistent with a reduction in specific torque. In addition, while control boys showed a progressive increase in specific torque production with advancing age, the DMD group did not. These findings demonstrate that noninvasive MRI assessments of contractile and non-contractile tissue in skeletal muscle can provide valuable information about disease progression in boys with DMD. Future longitudinal studies should be considered to further examine the effect of disease progression on muscle morphology and specific torque production and validate the results presented in this cross-sectional study design.

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Figure 1.

Representative T1-weighted 3-D gradient-echo magnetic resonance images (TR/TE, 24/1.8) of the mid-thigh for 14-year-old boy with DMD (A and C) and 12-year-old healthy boy (B and D).

RF, rectus femoris; VL, vastus lateralis; VI, vastus intermedius, VM, vastus medialis, BFf, biceps femoris-short head; BFh, biceps femoris-long head; ST, semitendinosus; SM, semimembranosus; AM, adductor magnus; AL, adductor longus; Gr, gracilis; Sar, sartorius Scale=5 cm

Figure 2.

Method for determining non-contractile and contractile contents in the muscles of boys with DMD (B and D) and control (A and C). A and B: outline of region of interests (ROIs) on the sartorius (Sar) and gracilis (Gr) muscles (white) consisting of approximately 50% of muscle and subcutaneous fat and on the biceps femoris-long head (red). C and D: histogram (number of pixels versus signal intensity) of ROIs from A or C. Histogram in black line with two peaks was obtained from ROIs from Sar and Gr and histogram in red line with one peak was obtained from BFL.

Dotted lines showed threshold calculated from maximum entropy method to divide contractile and non-contractile contents. Scale=5 cm

Figure 3. Contractile CSA (A) and non-contractile content (B) in QF, HM, AD and Total thigh muscles for DMD group and CONT group.
 *, $p < 0.05$; †, $p < 0.01$; #, $p < 0.001$
 QF, quadriceps femoris; HM, hamstrings; AD, hip adductors; Total, total thigh muscles measured

Figure 4.

Non-contrastile content comparison among categorized age groups in DMD.

BFI, biceps femoris-long head; RF, rectus femoris; AM, adductor magnus; Gr, gracilis.

*, $p < 0.02$ versus <8 years age group. a, $p < 0.02$ versus BFI.

<8 years age group included boys with DMD under-8-years-old ($n = 8$); 8 to <10 years age group included boys with DMD older than 8 years but younger than 10 years ($n = 7$); 10 to <12 years age group included boys with DMD older than 10 years but younger than 12 years ($n = 9$); ≥12 years age group included boys with DMD 12 years older ($n = 4$).

Figure 5.

Relationship between functional test and non-contractile content (%) in boys with DMD. Note that in Figs. B & C non-ambulatory boys are displayed as triangles, and these subjects were assigned a functional scale 2SD higher than the lowest performance (longest time) on each of the tasks. The assigned values in the non-ambulatory subjects were not included in the correlations analyses and are displayed for visual purpose only. HM, hamstrings.

Figure 6. Relationships between peak torque during isometric knee extension and contractile cross-sectional area (CSA) of the quadriceps femoris (A) and between specific torque and age (B).

Table 1

Subject demographics.

	DMD	CONT
Number of subjects	28	10
Number of ambulatory subjects	24	10
Age (years)	9.8 ± 0.4	11.4 ± 0.8
Height (cm)	129.1 ± 2.3*	147.3 ± 5.0
Weight (kg)	33.8 ± 2.4	38.6 ± 3.7
BMI (kg/m ²)	19.6 ± 0.8	17.4 ± 0.8

Values are mean ± SEM. DMD, Duchenne muscular dystrophy group; CONT, control group.

BMI, body mass index.

*, p < 0.05 versus CONT group.

Table 2

Non-contractile content, contractile CSA and non-contractile CSA of the thigh muscles for the DMD and CONT groups.

	Contractile CSA (cm ²)		Non-contractile CSA (cm ²)		Non-contractile content (%)	
	DMD	CONT	DMD	CONT	DMD	CONT
Quadriceps femoris						
RF	4.0 ± 0.4	5.2 ± 0.6	1.7 ± 0.4 [†]	0.1 ± 0.0	24.4 ± 5.3 [†]	1.2 ± 0.5
VL	11.4 ± 0.9	14.7 ± 1.5	2.0 ± 0.6	0.4 ± 0.1	15.6 ± 4.5	2.2 ± 0.6
VI	6.7 ± 0.6 [#]	13.0 ± 1.3	1.3 ± 0.4	0.0 ± 0.0	15.4 ± 4.8 [*]	0.7 ± 0.3
VM	3.2 ± 0.3 [#]	7.2 ± 0.8	0.7 ± 0.3	0.1 ± 0.0	14.6 ± 4.6 [*]	1.0 ± 0.4
Hamstrings						
BFs	0.5 ± 0.1 [†]	0.9 ± 0.1	0.0 ± 0.0	0.0 ± 0.0	9.9 ± 3.7	0.9 ± 0.4
BFL	4.6 ± 0.5	6.1 ± 0.5	3.1 ± 0.8 [*]	0.2 ± 0.1	32.5 ± 6.7 [*]	2.8 ± 0.7
ST	6.0 ± 0.4	5.2 ± 0.6	0.7 ± 0.2 [*]	0.1 ± 0.0	11.2 ± 3.4	2.1 ± 0.7
SM	2.4 ± 0.3	2.6 ± 0.5	1.0 ± 0.4	0.1 ± 0.0	19.8 ± 5.2 [*]	1.7 ± 0.7
Hip adductors						
AL	1.6 ± 0.2	2.5 ± 0.4	0.2 ± 0.1	0.0 ± 0.0	7.7 ± 3.3	1.1 ± 0.5
AM	6.1 ± 0.8 [#]	14.3 ± 1.1	2.4 ± 0.6 [*]	0.2 ± 0.1	29.4 ± 6.5 [†]	1.5 ± 0.7
Sar	2.4 ± 0.2	1.9 ± 0.2	0.2 ± 0.1	0.1 ± 0.0	7.0 ± 3.3	3.5 ± 1.1
Gr	3.1 ± 0.2	2.3 ± 0.2	0.1 ± 0.0	0.0 ± 0.0	3.3 ± 0.8	2.1 ± 0.7

Values are mean ± SEM. DMD, Duchenne muscular dystrophy group; CONT, control group; CSA, cross-sectional area; RF, rectus femoris; VL, vastus lateralis; VI, vastus intermedius; VM, vastus medialis; BFs, biceps femoris-short head; BFL, biceps femoris-long head; ST, semitendinosus; SM, semimembranosus; AL, adductor longus; AM, adductor magnus; Sar, sartorius; Gr, gracilis.

^{*}, p < 0.05;

[†], p < 0.01;

[#], p < 0.001 versus CONT group.

Table 3

Spearman's rank correlation coefficients between non-contractile content in each thigh muscle group and functional abilities in DMD group.

	n	DMD			
		QF	HM	AD	Total
30ft Walk	24	0.36	0.43 [*]	0.42 [*]	0.42 [*]
Supine Up	21	0.56 [†]	0.79 [†]	0.66 [†]	0.68 [†]
Chair Up	24	0.41 [*]	0.49 [*]	0.41 [*]	0.45 [*]
TUG	24	0.43 [*]	0.60 [†]	0.44 [*]	0.55 [†]
4 Stairs	24	0.49 [*]	0.56 [†]	0.57 [†]	0.56 [†]
Brooks scale	28	0.71 [†]	0.71 [†]	0.77 [†]	0.75 [†]

^{*}, p < 0.05;

[†] p < 0.01. DMD, Duchenne muscular dystrophy group.

QF, quadriceps femoris; HM, hamstrings; AD, adductors; Total, total thigh muscles.