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# Muscle architecture is associated with muscle fat replacement in Duchenne and Becker muscular dystrophies

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## Abstract

**Introduction/Aims:** Duchenne and Becker muscular dystrophies (DMD and BMD, respectively) are characterized by fat replacement of different skeletal muscles in a specific temporal order. Given the structural role of dystrophin in skeletal muscle mechanics, muscle architecture could be important in the progressive pathophysiology of muscle degeneration. Therefore, the aim of this study was to assess the role of muscle architecture in the progression of fat replacement in DMD and BMD.

**Methods:** We assessed the association between literature-based leg muscle architectural characteristics and muscle fat fraction from 22 DMD and 24 BMD patients. Dixon-based magnetic resonance imaging estimates of fat fractions at baseline and 12 (only DMD) and 24 months were related to fiber length and physiological cross-sectional area (PCSA) using age-controlled linear mixed modeling.

**Results:** DMD and BMD muscles with long fibers and BMD muscles with large PCSAs were associated with increased fat fraction. The effect of fiber length was stronger in muscles with larger PCSA.

**Discussion:** Muscle architecture may explain the pathophysiology of muscle degeneration in dystrophinopathies, in which proximal muscles with a larger mass (fiber length  $\times$  PCSA) are more susceptible, confirming the clinical observation of a temporal proximal-to-distal progression. These results give more insight into the mechanical role in the pathophysiology of muscular dystrophies. Ultimately, this new information can be used to help support the selection of current and the development of future therapies.

## KEYWORDS

dystrophin, fat fraction, MRI, muscle degeneration, pathophysiology

**Abbreviations:** AM, adductor magnus; BFL, biceps femoris long head; BMD, Becker muscular dystrophy; CI, confidence interval; DMD, Duchenne muscular dystrophy; EDL, extensor digitorum longus; FF, fat fraction; GL, gastrocnemius lateralis; GM, gastrocnemius medialis; GR, gracilis; L<sub>r</sub>, fiber length; logFF, logit transformation of the FF data; MRI, magnetic resonance imaging; N<sub>p</sub>, total number of pixels of the muscle ROI per slice; PCSA, physiological cross-sectional area; PER, peroneus; RF, rectus femoris; ROI, region of interest; SAR, sartorius; SI, signal intensity; ST, semitendinosus; SM, semimembranosus; SOL, soleus; TA, tibialis anterior; TP, tibialis posterior; VI, vastus intermedius; VL, vastus lateralis; VM, vastus medialis.

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## 1 | INTRODUCTION

Duchenne and Becker muscular dystrophies (DMD and BMD, respectively) are X-linked recessive diseases caused by mutations in the *DMD* gene leading to the absence (in DMD) or dysfunction (in BMD) of the dystrophin protein. The diseases are characterized by progressive muscle weakness associated with a gradual increase in the volume fraction of fat in muscle, and the presence of muscle inflammation and fibrosis.<sup>1</sup> The progression of fat replacement in different muscles exhibits a typical temporal order,<sup>2,3</sup> where, in general, the proximal muscles degenerate before the distal ones.<sup>4</sup> The pathophysiology of the muscle degeneration in DMD and BMD is poorly understood and it is also unknown why specific muscles are more susceptible to degeneration than others. The identification of factors that affect muscle preservation could support the selection of current and the development of future therapies.

Dystrophin is a vital part of the costamere, which connects the muscle fiber to the sarcolemma, providing mechanical stability within the muscle fiber.<sup>5-7</sup> The role of the costamere is to protect the contractile element from damage during dynamic contractions<sup>5-7</sup> and to shunt the contractile forces from the contractile filaments to the sarcolemma, resulting in fast force propagation over the muscle fiber.<sup>8</sup>

The spatial distribution of sarcomeres over the muscle determines the dynamic properties of the muscle. The number of serial-organized sarcomeres defines the muscle fiber length and potential contraction velocity, whereas the number of parallel-organized sarcomeres determines the muscle physiological cross-sectional area (PCSA) and the maximum muscle force.<sup>9</sup> Given the differences in muscle architecture and the structural role of dystrophin in the mechanics of the muscle fiber, muscle architecture may be an important property in the temporal order of fat replacement. Muscles with long fibers may be more susceptible to damage because of a higher inhomogeneity of instantaneous force distribution within the muscle fibers during activation.<sup>10</sup> Muscles with a larger PCSA could be more susceptible to damage because the load on muscle fibers can be higher during vigorous contractions. At submaximal loading, however, a large PCSA may be protective against damage because of relatively low fiber stresses, as the same force is divided over a larger area.

Magnetic resonance imaging (MRI) is ideally suited to assess the temporal order of muscle fat replacement in muscle disease. Several quantitative MRI studies in DMD and BMD have yielded important information about which muscles in the leg are susceptible to fat replacement early in the disease, and which muscles are relatively spared.<sup>2,11-15</sup> In DMD, this order has been associated with the amount of eccentric contraction during walking.<sup>16</sup> However, the correlation was only present when excluding two muscles from the analysis. The authors applied simple linear regression analyses and a cross-sectional design and therefore could not correct for the effect of age. A major disadvantage of simple linear regression analyses in a progressive disease such as a muscular dystrophy is that, because fat fraction (FF) increases with age, any variable correlating with age correlates with FF as well.<sup>17</sup>

In this study we aimed to identify the association between the increase in FF in DMD and BMD over time and fiber length and PCSA, while controlling for the effect of age. We hypothesized that fiber length and PCSA are associated with FF.

## 2 | METHODS

### 2.1 | MRI data acquisition

The DMD MRI data set used in this study was described by Hooijmans et al.<sup>18</sup> and Naarding et al.,<sup>17</sup> and the BMD data set by Hooijmans et al.<sup>19</sup> In short, male DMD and BMD patients were scanned at baseline and after about 1 (only DMD) and 2 years. All patients were recruited from the Dutch Dystrophinopathy Database and diagnosis was confirmed by genetic testing.<sup>20</sup> Our study was approved by the local medical ethics committee. Written informed consent was given by all patients and/or their parents.

### 2.2 | MRI acquisition and analysis

MRI examinations were performed on a 3T MR scanner (Ingenia; Philips Healthcare, Best, The Netherlands) and included a 3-point gradient-echo Dixon sequence (23 slices; voxel size:  $1 \times 1 \times 10$  mm; slice gap: 5 mm; repetition time [TR]/echo time [TE]/echo time shift [ $\Delta$ TE]: 210/4.41/0.76 milliseconds; 2 averages; flip angle [FA]:  $8^\circ$ ) to assess muscle fat replacement in the upper and lower leg muscles. The scans were aligned perpendicular to the femur and tibia.

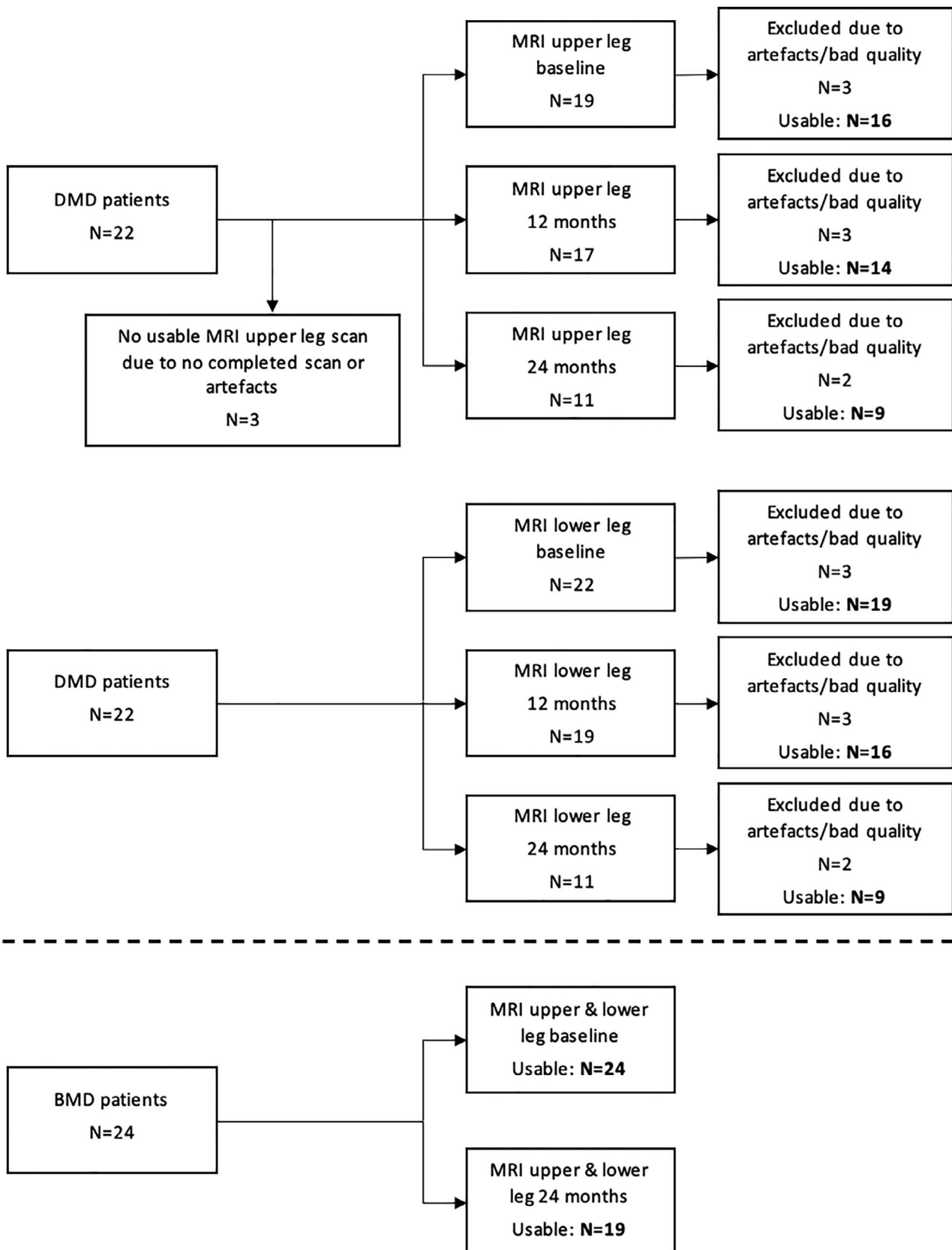
Water and fat images were reconstructed offline using the same in-house water-fat separation algorithm as Hooijmans et al.,<sup>19</sup> built in MATLAB 2019b (The MathWorks, Natick, MA). This algorithm was based on the known frequencies of a six-peak lipid spectrum.<sup>21</sup>

The images were visually checked for bulk motion artifacts and were excluded if these were present. Regions of interest (ROIs) were drawn in five slices for ten upper and seven lower leg muscles using the Medical Image Processing, Analysis and Visualization program (<http://mipav.cit.nih.gov>). The most proximal slice with the biceps femoris short head still visible was chosen to be the center of the five upper leg slices; the thickest part of the calf was chosen to be the center for the five lower leg slices. The ten upper leg muscles consisted of the rectus femoris (RF), vastus medialis (VM), vastus lateralis (VL), vastus intermedius (VI), biceps femoris long head (BFL), semitendinosus (ST), semimembranosus (SM), adductor magnus (AM), gracilis (GR), and sartorius (SAR). The seven lower leg muscles were the gastrocnemius medialis (GM), gastrocnemius lateralis (GL), soleus (SOL), tibialis anterior (TA), tibialis posterior (TP), extensor digitorum longus (EDL), and peroneus (PER). Fat replacement was quantified by the fat fraction per muscle (FF) and per slice  $i$  using the signal intensity of fat ( $SI_{\text{FAT}}$ ) from the fat image and water ( $SI_{\text{WATER}}$ ) from the separate water image (Equation 1):

$$FF(i) = \frac{SI_{\text{FAT}}(i)}{SI_{\text{FAT}}(i) + SI_{\text{WATER}}(i)} \quad (1)$$

Subsequently, a weighted average of the five slices was calculated per muscle (Equation 2):

$$FF = \frac{\sum (FF(i) * N_P(i))}{\sum N_P(i)} \text{ for } i = (1 - 5) \text{ slices} \quad (2)$$



**FIGURE 1** Details on the inclusion of DMD and BMD patients and longitudinal upper and lower leg MRI scans. For DMD, in total 39 usable data sets were available for the upper leg and 44 for the lower leg. For BMD, 43 usable data sets were available for the upper and lower leg. Abbreviations: BMD, Becker muscular dystrophy; DMD, Duchenne muscular dystrophy; MRI, magnetic resonance imaging

where  $N_p(i)$  is the total number of pixels of the muscle ROI per slice.

### 2.3 | Muscle characteristics

The PCSA and muscle fiber length ( $L_f$ ) of adult muscles were extracted from the data reported by Ward et al.<sup>22</sup> The reported PCSAs contain an additional cosine factor to be able to relate PCSA to force (Equation 3), and do not represent the actual PCSAs describing surface of parallel sarcomeres and estimated by muscle volume over fiber length.<sup>23</sup>

$$\frac{\text{Muscle mass [g]} \cdot \cos(\theta [^\circ])}{\text{Fiber length [cm]} \cdot \rho \left[ \frac{\text{g}}{\text{cm}^3} \right]} \quad (3)$$

where  $\theta$  is the pennation angle and  $\rho$  is the muscle density of 1.056 g/cm<sup>3</sup>. Therefore, we derived the “classical” PCSA by dividing the PCSA values of Ward et al.<sup>22</sup> by the cosine of the pennation angles, reported in same study, and included the classical values in our analyses.

### 2.4 | Statistical analysis

For every patient we acquired data from two or three time-points and multiple muscles. A linear mixed-effects model was used to analyze the relation between muscle characteristics and muscle fat replacement. The model and graphs were built using R software (R Core Team, 2019) in combination with the packages *lme4*<sup>24</sup> and *visreg*.<sup>25</sup> The full code is publicly available at Github (<https://git.lumc.nl/neuroscience/2021-veegertj-lmem-in-dmd-bmd>; using version at commit 4924b94c).

The FF is limited between 0 and 1 and thus not normally distributed. In addition, the age-dependent FF follows an S-shaped “logistic growth” curve rather than a linear relationship.<sup>17,26</sup> To obtain a normal distribution and a linear relationship between FF and age, a logit transformation of the FF data (logFF) was applied:

$$\log\text{FF} = \ln\left(\frac{\text{FF}}{1 - \text{FF}}\right) \quad (4)$$

The linear mixed-effects model design also included the mean-centered variables of age (years),  $L_f$  (cm), and PCSA (cm<sup>2</sup>), and an interaction term ( $L_f \times \text{PCSA}$ ) for each of the 17 leg muscles as predictor (fixed) variables. To account for varying disease progression and onset between patients and muscles, random effects were included according to the design with the highest log-likelihood value, being a by-patient and by-muscle random intercept and slope for the effect of age.

$P$  values were obtained by  $t$  tests using the *lmerTest* package<sup>27</sup> and significance was set at  $P < .05$ . The Holm-Bonferroni correction was applied to adjust for multiple comparisons (ie, for each of the four fixed effects).<sup>28</sup>

**TABLE 1** Descriptive statistics

	DMD	BMD
Patients, n	22	24
Age, years		
Mean (SD)	9.9 (3.0)	41.4 (12.8)
Range	6.5-17.4	18.8-68.2
Height (cm), mean (SD)	137.1 <sup>a</sup> (20.9)	179.1 <sup>a</sup> (7.6)
Weight (kg), mean (SD)	45.3 <sup>a</sup> (22.2)	77.0 <sup>a</sup> (12.4)
Lost ambulation, n		
Baseline	9	0
12 months	10	-
24 months	10	1
Fat fraction, mean (SD)		
Upper leg	0.453 (0.260)	0.441 (0.310)
Lower leg	0.310 (0.210)	0.245 (0.206)

Abbreviations: BMD, Becker muscular dystrophy; DMD, Duchenne muscular dystrophy; SD, standard deviation.

<sup>a</sup>Not available for all patients.

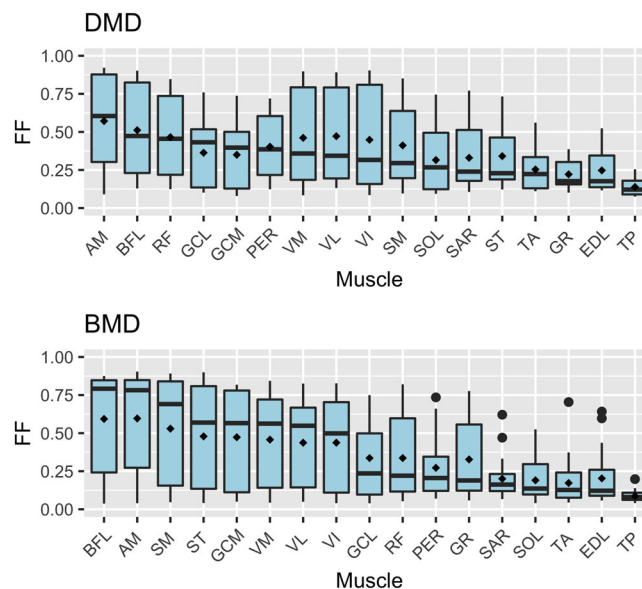
## 3 | RESULTS

For three DMD patients, no usable upper leg MRI scans were acquired at any of the three timepoints, either due to image artifacts or because the patient could not complete the scan (Figure 1). In total, 39 MRI scans were available for the upper leg, and 44 for the lower leg, over all three time-points (see Table 1 for descriptive statistics and Tables S1 and S2 for an overview of mutations). For BMD, all data were included and, in total, 43 MRI scans were available for both upper and lower leg over the two time-points (Figure 1).

The median FF of individual muscles for all DMD and BMD patients at baseline (Figure 2) showed that the AM and BFL had the highest FFs, with AM highest in DMD and BFL in BMD, and that the EDL and TP had the lowest FFs.

An association between age and logFF was observed in DMD and BMD (Table 2), with higher age corresponding to higher logFF (positive slope).  $L_f$  was also associated with logFF for both DMD and BMD, showing that muscles with longer fibers were associated with a higher logFF. By contrast, PCSA was significantly associated with logFF only in BMD patients. The interaction  $L_f \times \text{PCSA}$  was significantly associated with logFF in both DMD and BMD, where the effect of  $L_f$  on logFF was stronger if PCSA was larger. The association between age and logFF was stronger in DMD than BMD, whereas the other three associations,  $L_f$ , PCSA, and  $L_f \times \text{PCSA}$ , were stronger in BMD than DMD patients.

To visualize the separate effects of age,  $L_f$ , and PCSA on the logFF (Table 2), on the original FF scale, we fitted the predicted FF based on the three individual predictors, while keeping the other two predictors at the median value. This resulted in the six graphs in Figure 3. For example, the top two panels represent the effect of age (x axis) on FF (y axis) for a muscle with a median  $L_f$  and PCSA. The



**FIGURE 2** FF per muscle over all DMD (upper panel) and BMD (lower panel) patients for the baseline measurement. Lines within boxes indicate the median value and define the order on the x axis, black diamonds represent the mean, and the lower and upper bounds indicate the first and third quartiles. Upper whiskers show the largest value with, as maximum,  $1.5 \times$  IQR, and lower whiskers show the smallest value with, as minimum,  $1.5 \times$  IQR. Individual round dots represent outliers. Muscles are ordered from highest median FF on the left to lowest median FF on the right. Abbreviations: AM, adductor magnus; BFL, biceps femoris long head; BMD, Becker muscular dystrophy; DMD, Duchenne muscular dystrophy; EDL, extensor digitorum longus; FF, fat fraction; GL, gastrocnemius lateralis; GM, gastrocnemius medialis; GR, gracilis; IQR, interquartile range; MRI, magnetic resonance imaging; PER, peroneus; RF, rectus femoris; SAR, sartorius; ST, semitendinosus; SM, semimembranosus; SOL, soleus; TA, tibialis anterior; TP, tibialis posterior; VI, vastus intermedius; VL, vastus lateralis; VM, vastus medialis

	<i>B</i> (a.u.) <sup>a</sup>	<i>SE B</i> (a.u.) <sup>a</sup>	95% CI (a.u.) <sup>a</sup>	<i>P</i> value <sup>b</sup>
<b>DMD</b>				
Age (years)	0.281	0.055	0.167-0.394	<.001 <sup>c</sup>
<i>L<sub>f</sub></i> (cm)	0.052	0.018	0.007-0.097	.030 <sup>c</sup>
PCSA (cm <sup>2</sup> )	0.011	0.008	-0.010 to 0.033	.151
<i>L<sub>f</sub></i> × PCSA (a.u.)	0.003	0.001	0.000-0.006	.034 <sup>c</sup>
<b>BMD</b>				
Age (years)	0.031	0.015	0.013-1.250	.049 <sup>c</sup>
<i>L<sub>f</sub></i> (cm)	0.114	0.027	1.130-3.437	.002 <sup>c</sup>
PCSA (cm <sup>2</sup> )	0.052	0.012	0.526-1.540	.002 <sup>c</sup>
<i>L<sub>f</sub></i> × PCSA (a.u.)	0.007	0.002	1.426-4.432	.002 <sup>c</sup>

**TABLE 2** Output linear mixed effects model

Abbreviations: CI, confidence interval; *L<sub>f</sub>*, fiber length; PCSA, physiological cross-sectional area; SE, standard error.

<sup>a</sup>Values presented in the log odds-transformed scale and correspond to the mean-centered predictors.

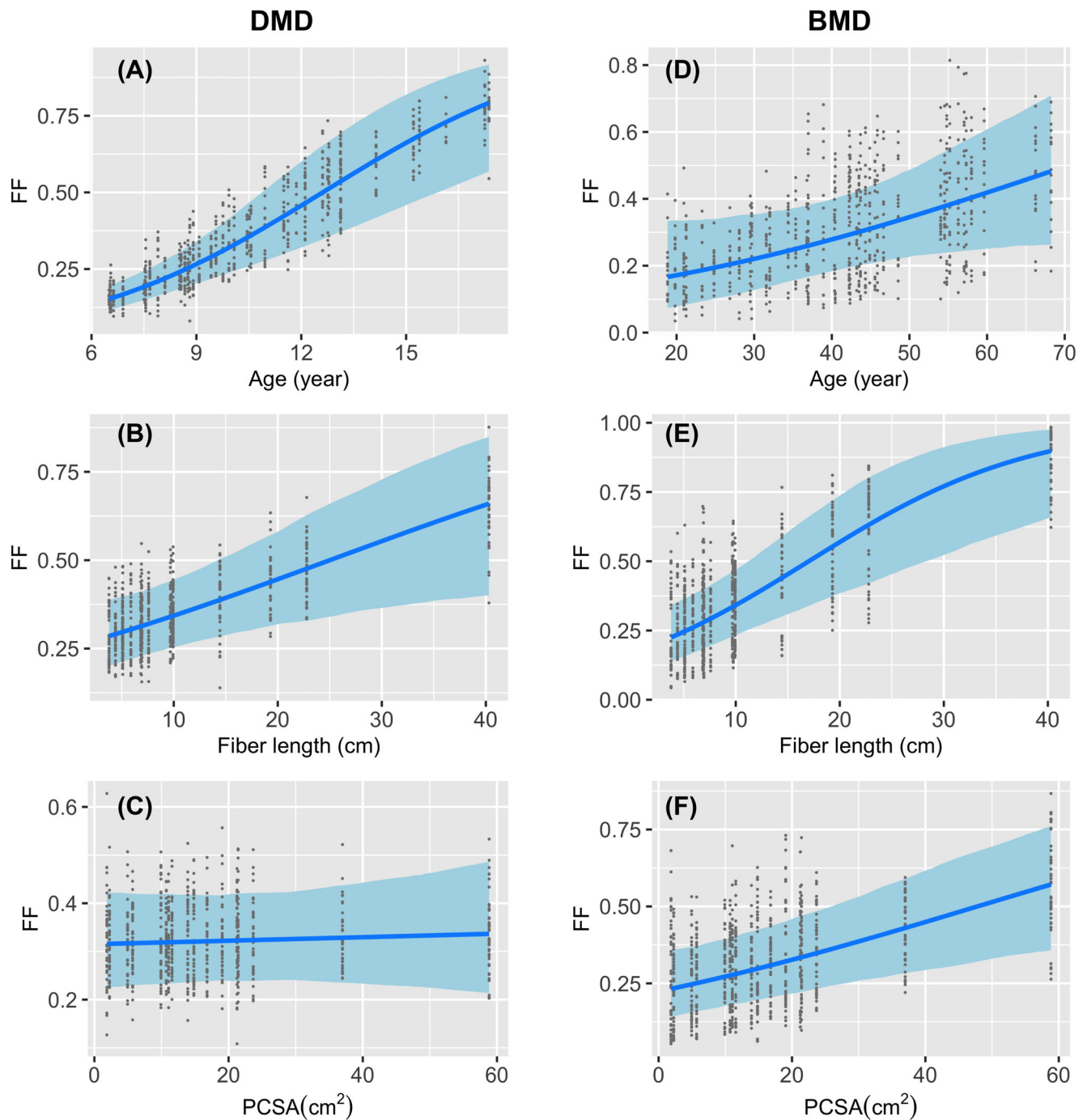
<sup>b</sup>Values adjusted using the Holm-Bonferroni method.

<sup>c</sup>Statistically significant, *P* < .05.

graphs show an association for DMD of FF with age and *L<sub>f</sub>*, but not with PCSA, whereas, for BMD, FF was associated with all three predictors.

In Figure 4, the interaction effect *L<sub>f</sub>* × PCSA is illustrated. To this end, the FF was predicted for every combination of *L<sub>f</sub>* and PCSA for a patient with a median age. The graphs show that the interaction effect is stronger in BMD than in DMD, as indicated

by the stronger curvature of the contours. In the DMD graph, the contours switch “direction” in the lower *L<sub>f</sub>* regions, where a larger PCSA leads to lower FF values for *L<sub>f</sub>* < 7 cm and higher FF values for *L<sub>f</sub>* > 7 cm. What also stands out is that the FF values are higher in BMD than in DMD, which can be explained by a higher average FF for a patient with the median age in BMD compared with DMD.

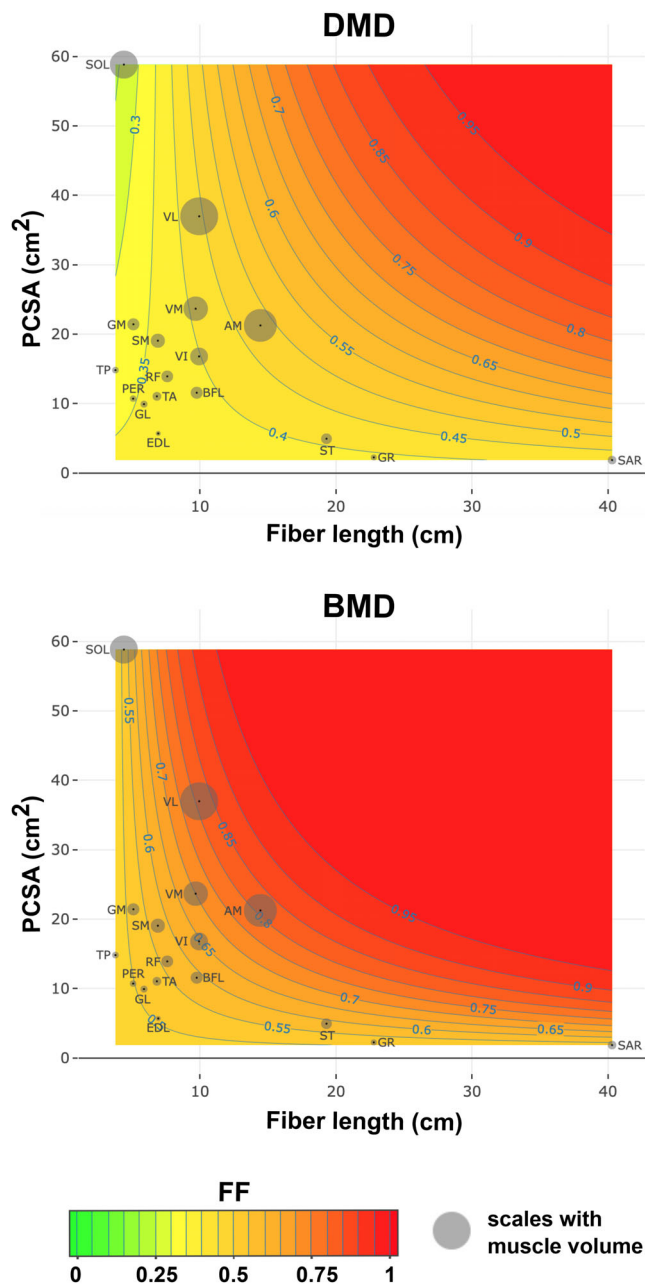


**FIGURE 3** Prediction fits (blue line) for the three fixed effects and their effect on (FF), while keeping the other two variables constant at the median value, with the 95% confidence intervals (blue bands) and the partial residuals for the 698 (DMD) and 731 (BMD) available data points (dots). The values are transformed back to the real FF scale. Abbreviations: BMD, Becker muscular dystrophy; DMD, Duchenne muscular dystrophy; FF, fat fraction; PCSA, physiological cross-sectional area

## 4 | DISCUSSION

Our data are in line with our hypothesis: When correcting for age, muscles with long fibers and the interaction between fiber length and PCSA were associated with high FF values in DMD and BMD. For BMD, a larger PCSA was significantly associated with a higher FF as well.

In the absence of dystrophin the inhomogeneity of force distribution within muscle fibers has been suggested to play a role in damage.<sup>10</sup> This hypothesis was corroborated with the observation that force propagation along the muscle fiber is slower in DMD patients.<sup>8</sup> Our results further support this hypothesis, as we have shown that muscles with long fibers are more susceptible to fat replacement in both DMD and BMD. Muscles with longer fibers are theoretically able



**FIGURE 4** Visual representation of the interaction between fiber length and PCSA for DMD (upper panel) and BMD (lower panel). The color scale indicates the predicted fat fraction (FF) for every combination of fiber length and PCSA for a patient with the median age (DMD  $\approx$  10 years, BMD  $\approx$  41 years). The contour lines are shown for every step of 0.05 FF. The muscles are located in the figure according to their fiber length and PCSA, the size of the circles is proportional to their mass as retrieved from Ward et al.<sup>22</sup> Abbreviations: DMD, Duchenne muscular dystrophy; FF, fat fraction; PCSA, physiological cross-sectional area

to reach a high contraction velocity, and it is likely that an impaired force propagation in such fibers increases the inhomogeneity in force distribution, possibly leading to contraction-induced damage.

In BMD, muscles that are theoretically able to produce a high force (large PCSA) also were associated with increased fat

replacement. This could be due to the absence or dysfunction of dystrophin impairing force transmission to the sarcolemma.<sup>7</sup> The interaction effect between fiber length and PCSA may also be interpreted as representative of muscle volume, because muscle volume can be estimated by fiber length multiplied by PCSA. Muscle volume is proportional to the maximum muscle power, independent of the organization of these sarcomeres.<sup>9</sup> Thus, this interaction suggests that muscles with both long fibers and a large PCSA, and therefore able to produce more power, are more susceptible to fat replacement. Because these muscles have a large volume, this is also in line with the clinical observation of a proximal-to-distal order of muscle degeneration.<sup>4</sup>

The directions of the associations we found were comparable between DMD and BMD, but the effect of age was stronger in DMD than in BMD and the effect size of the significant parameters was larger in BMD (Table 2 and Figure 4). The smaller effect of age is likely due to the slower and more variable disease progression in BMD, as the age of onset and progression of muscle damage and clinical symptoms can vary greatly.<sup>29,30</sup> The larger effect size of the associations for fiber length and PCSA could be related to the slower progression of BMD compared with DMD.<sup>29,30</sup> In DMD, muscle damage likely starts soon after birth, and patients show muscle weakness early in life.<sup>31</sup> In BMD, symptom onset is usually much later, and extensive muscle damage only becomes apparent when patients are older, when the loads on muscles are higher. This could result in fat replacement being more influenced by muscle architecture in BMD patients.

Some limitations of the study should be acknowledged. First, we chose to include only fiber length and PCSA as characteristics determining the dynamic properties of a muscle, whereas muscle mass, pennation angle, and fiber type composition also could play roles.<sup>9,32</sup> However, mass and pennation angle are highly dependent on fiber length and PCSA and would therefore add complexity to the model without significant additional information. Fiber type composition in human muscles, however, shows wide variation between individuals and heterogeneity within muscles.<sup>33,34</sup> Moreover, recent literature has described the existence of hybrid muscle fibers and questioned the classical idea of purely fast and slow fibers.<sup>35</sup> Therefore, reliable fiber type composition data sets including all leg muscle are not available. Second, we extracted the muscle characteristics from the study by Ward and colleagues<sup>22</sup> based on adult cadavers, whereas the DMD patients included in this study were, on average, 9.9 years old, ranging from 6.5 to 17.4 years at baseline. Therefore, the absolute values of the muscle characteristics may not be representative of the actual values for this cohort. However, proportions between body segments of children 8 years of age have been shown to be like those of adults<sup>36</sup> and were therefore not expected to have a major impact on the associations found in this study. The average age of the BMD patients was 41 years and the associations found in these patients were similar and even stronger than for the DMD patients. Therefore, it is possible that if we would have been able to include data on muscle characteristics of children, the associations would have been even stronger.

In this study we chose to include data of both DMD and BMD patients because they share the same pathological background, a mutation of the *DMD* gene. The fact that the resulting associations between muscle architecture and fat fraction were comparable supports the hypothesis that muscle architecture indeed plays a role in the pathophysiology in these diseases. It would be of interest to perform the same analysis on other muscular dystrophies with affected proteins in other parts of the dystrophin-glycoprotein complex or with a completely different pathological background. In R9 dystroglycan-related limb-girdle muscular dystrophy, for example, a slightly different pattern of muscle involvement compared with DMD and BMD can be observed, although the affected protein is also part of the dystrophin-glycoprotein complex.<sup>37</sup> The pattern seems to show an earlier involvement of the short-fibered SM with larger PCSA and longer fibered ST with relatively small PCSA and a later involvement of the short-fibered RF with an average PCSA, when compared with DMD and BMD. Our mixed effects model allows assessment of the role of muscle architecture, but inherently leaves part of the variance unexplained. Therefore, even with differences in muscle involvement, a role of muscle architecture should not be excluded. In addition, although proteins are part of the dystrophin-glycoprotein complex, they may have distinct roles; for example, it is known that the complex plays an important role in multiple nonmechanical signaling pathways.<sup>38</sup> In a disease such as facioscapulohumeral muscular dystrophy,<sup>39-41</sup> with a different pathophysiology, the mechanical interactions between the contractile proteins and the sarcolemma are intact<sup>42</sup> and structural and mechanical properties may be of less importance.

In conclusion, an association between muscle architecture and FF in DMD and BMD patients was observed. Muscles with longer muscle fibers were associated with higher FF values, and this effect was stronger if the PCSA of the muscle was larger. For BMD, a larger PCSA was also significantly associated with a higher FF. Our results indicate that larger muscles are more susceptible to fat replacement than small ones, confirming the clinical observation of a proximal-to-distal order of muscle degeneration. These results give more insight in the mechanical role in the pathophysiology of muscular dystrophies. Future longitudinal studies could explore the potential use of this information to track disease progression and responses to therapies.

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#### CONFLICT OF INTEREST

E.H.N. reports ad hoc consultancies for WAVE, Santhera, Regenxbio, and PTC, and has worked as an investigator in clinical trials involving Italfarmaco, NS Pharma, Reveragen, Roche, WAVE, and Sarepta outside the submitted work. All reimbursements were received by the LUMC. No personal financial benefits were received. The remaining authors have no conflicts of interest.











#### ETHICAL PUBLICATION STATEMENT

We confirm that we have read the Journal's position on issues involved in ethical publication and affirm that this report is consistent with those guidelines.

#### DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available on request from the corresponding author. The data are not publicly available due to privacy or ethical restrictions.

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## SUPPORTING INFORMATION

Additional supporting information may be found in the online version of the article at the publisher's website.

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