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Skeletal muscle imaging in facioscapulohumeral muscular dystrophy, pattern and asymmetry of individual muscle involvement

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Abstract

To better understand postural and movement disabilities, the pattern of total body muscle fat infiltration was analyzed in a large group of patients with facioscapulohumeral muscular dystrophy. Additionally, we studied whether residual D4Z4 repeat array length adjusted for age and gender could predict the degree of muscle involvement. Total body computed tomography scans of 70 patients were used to assess the degree of fat infiltration of 42 muscles from neck to ankle level on a semi-quantitative scale. Groups of muscles that highly correlated regarding fat infiltration were identified using factor analysis. Linear regression analysis was performed using muscle fat infiltration as the dependent variable and D4Z4 repeat length and age as independent variables. A pattern of muscle fat infiltration in facioscapulohumeral muscular dystrophy could be constructed. Trunk muscles were most frequently affected. Of these, back extensors were more frequently affected than previously reported. Asymmetry in muscle involvement was seen in 45% of the muscles that were infiltrated with fat. The right-sided upper extremity showed significantly higher scores for fat infiltration compared to the left side, which could not be explained by handedness. It was possible to explain 29% of the fat infiltration based on D4Z4 repeat length, corrected for age and gender. Based on our results we conclude that frequent involvement of fat infiltration in back extensors, in addition to the abdominal muscles, emphasizes the extent of trunk involvement, which may have a profound impact on postural control even in otherwise mildly affected patients.

Keywords: Fatty infiltration; FSHD; Muscle imaging; Neuromuscular disorders; Computer tomography

1. Introduction

Facioscapulohumeral muscular dystrophy (FSHD) is a common form of muscular dystrophy, with a clinical presentation of progressive weakness in the muscles of the face, shoulder girdle, upper arm, trunk and lower limbs [1]. The extent of muscle abnormalities can be variable both between subjects and between different muscles of the body [2–4]. Disease progression is also variable, with asymptomatic patients suffering from the least severe form and patients with infantile onset and early wheelchair dependency from the most progressive form [2,4]. Disease severity is thought to be partly related to the genetic defect, i.e. the length of the residual D4Z4 repeat array [5–7].

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Previously, we have shown that falling is a major problem in FSHD [8,9]. In this paper we investigate the pattern of total body muscle fat infiltration to better understand which muscles might be critical for causing postural instability. Until now, the pattern of muscle involvement in FSHD has been described mainly by the clinical manifestations of muscle weakness [6,10,11]. However, not all muscles can be selectively evaluated using physical testing. Muscle imaging, on the other hand, enables the selective assessment of all body muscles including those that are hard to assess clinically.

Only two studies have described the pattern of muscle involvement in groups of up to 24 patients with FSHD in relation to genetic defect size [3,12]. These studies yielded contradicting results regarding the most frequently involved muscles and associations with genetic defect size and muscle function. Wang et al. [12] found that the hamstrings were the most severely affected muscles in the lower limbs, whereas Olsen et al. [3] reported that the tibialis anterior as well as the hamstring muscle semimembranosus were most specifically affected. Furthermore, associations between computed tomography (CT) grades for fat infiltration and D4Z4 fragment size reported by Wang et al. [12] were not corroborated by Olsen et al. [3]. Asymmetry scores reported by both studies were comparable but low considering the clinical observations of characteristic asymmetric muscle involvement [10,11,13,14]. Unfortunately, Olsen et al. [3] only assessed muscle involvement of the lower extremity.

Because of the few and inconsistent results, this study reports the results of CT muscle imaging from neck to ankle level in a large group of 70 well characterized FSHD patients using a systematic approach [15]. To better understand postural and movement disabilities in FSHD, we aimed to analyze the pattern of individual muscle involvement. We also aimed to determine the extent of asymmetry and to relate the degree of muscle involvement to muscle strength as well as to genetic defect size, corrected for age and gender.

2. Methods

2.1. Subjects

Seventy patients with FSHD who participated in a previously published randomized controlled trial were included in this study [15]. They were recruited via the Dutch Neuromuscular Diseases Association and the Neuromuscular Centre Nijmegen. The diagnosis FSHD was genetically confirmed as all included patients, or a first-degree relative, had a D4Z4 contraction at 4q35 with *Eco*RI fragments smaller than 38 kb after double digestion with *Bln*I [16,17]. Self-reported handedness was derived from a questionnaire. The study was approved by the Committee on research involving Human Subjects in Nijmegen, The Netherlands. Informed consent was obtained from all subjects.

2.2. Computed tomography

CT scans were made in a systematic way [18] to analyze changes in total body muscle volume as part of the previously reported trial [15]. Since most body muscles were visualized, baseline CT scans from this trial are valuable sources to assess the pattern of individual muscle involvement in FSHD. None of the participants had undergone any intervention at the time of the baseline scans. All data were collected before 2001. In short, subjects were scanned lying supine with their arms alongside the body. The first scan fell at an arbitrary position ranging from 0 till 9 cm caudally from the top of the scalp, followed by systematic equidistant sections (10 cm) in the transverse plane throughout the rostrocaudal axis of the body.

Fat infiltration was scored by a research assistant (RvA) who had been extensively trained to grade muscle involvement on CT scans. Of all 70 available CT scans fat infiltration was determined for 42 muscles on both the left and right body side, using the semi-quantitative four-point scale of Schwartz et al. [19]. Muscles were graded as: (1) normal appearance; (2) 'early moth-eaten' appearance (i.e. scattered areas of reduced attenuation); (3) 'late moth-eaten' appearance with many discrete areas of reduced attenuation and some confluent zones of abnormality; (4) 'washed out' appearance either with large confluent zones of low attenuation or complete replacement of the muscle by fatty tissue, with a rim of fascia remaining. Muscles were graded based on the most affected slice. Reliability of scoring was tested by blindly re-testing all muscles in a random sample of 10 subjects; an intraclass-correlation coefficient (ICC) of 0.95 was found. Examples of CT slices at shoulder, upper leg and lower leg regions are displayed in Fig. 1.

2.3. Muscle strength testing

For comparison of fatty infiltration grades with muscle strength, the Maximum Voluntary Isometric Contraction (MVIC) was assessed. Strength assessments were performed at the same day as the CT scans were made. Measurements were performed on a Quantitative Muscle Assessment testing system [15]. Shoulder abductors, elbow flexors, elbow extensors, knee extensors, knee flexors and ankle dorsal flexors were assessed. Muscles were tested on both body sides in a standardized way by one well-trained neurology resident (EvdK). The highest force (kg) of two maximal isometric contractions during 3–4 s was used for data analysis.

2.4. Statistical methods

Because of the non-linearity of the four-point scale, the CT grades were dichotomized into 'fat infiltration' (grades 2–4) versus no 'fat infiltration' (grade 1). The grades of identical muscles at the right and the left side of the

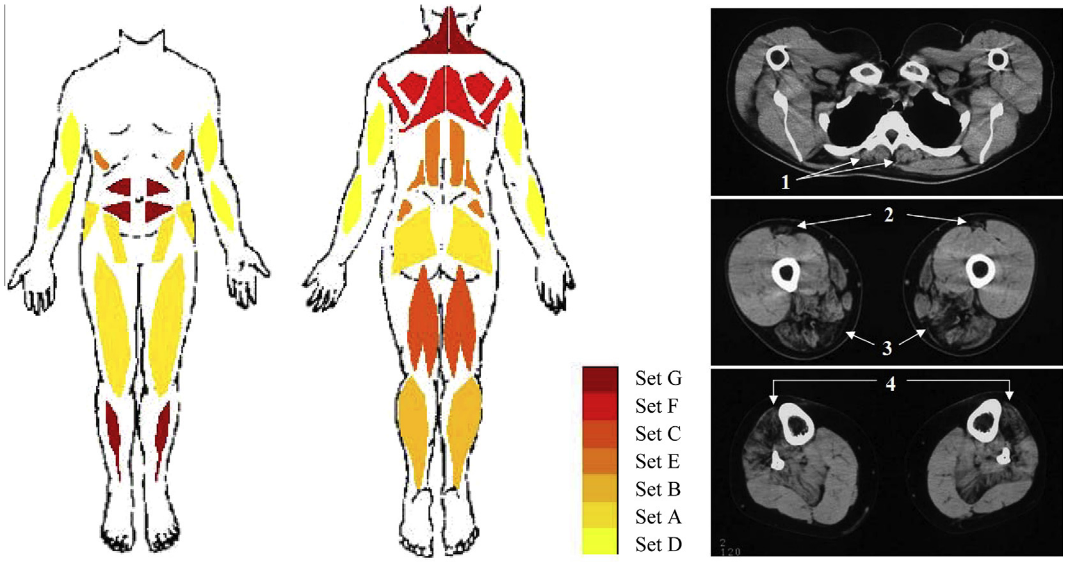

Fig. 1. On the left side, a visual representation of the factors (muscle groups ordered by degree of fatty infiltration) from red (most frequently affected) to yellow (least frequently affected). On the right side examples of three computed tomography (CT) slices at shoulder, upper leg, and lower leg level showing fatty infiltration in the mm. longissimus pars thoracale (1), mm. rectus femoris (2), hamstrings (3) and mm. tibialis anterior (4).

body were combined into one score. Fat infiltration was considered present if either side was affected. To identify the total body pattern of muscle fat infiltration, 'factors' were identified consisting of muscles that were highly correlated regarding the presence of fat infiltration. This was done by factor analysis using principal axis factoring as the extraction method. The varimax rotation with Kaiser normalization was used to simplify the interpretation of the factors. Extraction of the factors was based on the Kaiser's criterion for Eigenvalues being equal to or greater than 1.5. Cronbach's alpha was used to calculate the internal consistency of the constructed factors [20]. Muscles with more than 10 missing values were not included in the factor analysis.

Fat infiltration of an individual muscle was defined as 'asymmetric' if fat infiltration was found on one side while the contralateral side showed normal muscle tissue. The asymmetry score was graded as follows: +1, if fat infiltration was only found on the right side; 0, in the case of fat or no fat infiltration on both sides; −1, if fat infiltration was only found on the left side. The percentage asymmetry of three body parts (upper extremities, trunk and lower extremities) was calculated as the sum of the asymmetry scores of the muscles divided by the number of muscles of that body part (×100). A one sample *t*-test was used to separately test the percentage asymmetry of the total body as well as of each body part for statistical significance (i.e., difference from zero). The two samples *t*-test was used to test the difference in the percentage asymmetry between right handed and left handed patients, again for each body part separately. Additionally, asymmetry scores were calculated for differences in the degree of fat infiltration between the left and right body side, reflected by all differences in CT grades.

A linear regression model was constructed to study the influence of residual D4Z4 repeat length (kb) on the percentage of muscles with fat infiltration in each body part, adjusted for age (years) and gender (0 represents the male gender, 1 the female gender). The percentage fat infiltration of a body part was calculated as the number of muscles with fat infiltration divided by the number of muscles of that particular body part (×100).

To evaluate whether a higher CT grade was associated with decreased muscle strength, *Spearman* correlations coefficients were calculated. Shoulder abduction strength was correlated to CT grades of the m. deltoideus, elbow flexion strength to the m. biceps brachii, elbow extension strength to the m. triceps brachii, ankle dorsiflexion to the m. tibialis anterior, knee extension to the quadriceps muscles, and knee flexion strength to the CT grades of the hamstrings.

3. Results

3.1. Subjects

Patient characteristics are displayed in Table 1. The group of 70 patients consisted of 45 men and 25 women with a mean (±SD) age of 38 (±11) years. The average (±SD) D4Z4 repeat length was 26 (±6) kb. A wide range of disease severity was present; e.g., two patients showed only facial muscle weakness, whereas four patients made use of a wheelchair. All patients were able to walk 10 m, 37 of them made use of ankle–foot orthoses or orthopedic shoes. The self-reported handedness was

Table 1
Patient characteristics.

Male/female, *n*	45/25
Dominant hand (R/L)	63/6
Age, year	38 ± 11
Height, cm	180 ± 9
Weight, kg	76 ± 13
D4Z4 fragment size, kb	26 ± 6

Values for age, height, weight and D4Z4 fragment size are means ± SD.

right-sided for 63 and left-sided for 6 patients. One patient did not report preferred handedness.

3.2. Muscle involvement

Table 2 shows the percentage of subjects with fat infiltration on either side of the body for all 42 muscles assessed. In some patients it was not possible to properly assess smaller muscles; these were treated as missing values. Large differences in the frequency of fat infiltration were identified between individual muscles. In the upper extremity the deltoid muscle (68%) was most frequently affected, followed by the biceps brachii muscle (57%). In the trunk, more than half of the muscles were infiltrated with fat in nearly all patients (⩾85%). The trapezius muscle (97%) and the m. latissimus dorsi (94%) were most frequently affected. The m. semimembranosus was the most frequently affected muscle of the lower extremity (89%), followed by the anterior tibial muscle with a percentage of 71%.

3.3. Factor analysis

Factor analysis was performed using 37 of the 42 muscles. Five muscles were excluded from the analysis because of too many missing values (Table 2). The analysis resulted in seven factors explaining 61% of the total variance (based on 29 muscles). Each of the seven factors represents a group of muscles that match regarding the presence of fatty infiltration. Table 3 shows the muscles assigned to each specific factor. High Cronbach's alpha values were found for all factors (0.54–0.96), indicating good internal consistencies. Fig. 2 shows the ordering of the factors based on frequency of involvement (of at least one muscle within the factor). The four most frequently involved factors (muscle groups) were afflicted in more than 90% of the subjects. In Fig. 1 the pattern of muscle fat infiltration is visualized. This figure shows the most frequently involved muscles in red, while the least frequently involved muscles is displayed in yellow.

3.4. Asymmetry

Table 4 shows the percentage asymmetric fat infiltration by body part. We found that the muscles of the right upper extremity were, as a percentage, significantly more often infiltrated with fat compared to the left side (Δ7.0% (95% CI: 2.4; 11.6)). A similar result was found for the right-handed patients only (Δ7.4% (95% CI: 2.4; 12.4)). Nevertheless, the mean CT grade was not significantly different for the upper extremity muscles on either body side. For the left-handed patients no difference in the frequency or degree of fat infiltration between the right and left upper extremity was found (Δ3.3% (95% CI: −5.2; 12.0)). The percentage of individual muscle involvement for upper extremity muscles is described in Table 5. Of all muscles infiltrated with fat, 23% showed asymmetric involvement. When asymmetry was calculated taking into account also the differences in CT grades, a whole body asymmetry score of 45% was found.

3.5. Muscle strength

Table 6 shows the average strengths of the muscles grouped by CT grade for both the left and the right side of the body. For nearly all movements strong and significant (inverse) correlations were found between CT muscle involvement and muscle strength on both sides of the body. Only for shoulder abduction and deltoid CT grade, correlations lower than $r_s = 0.45$ were found.

3.6. D4Z4 constriction and muscle fat infiltration

Corrected for age and gender, we found statistically significant (inverse) relations between the D4Z4 repeat length and the percentage of fat infiltrated muscles of the trunk ($R^2 = .19$ (β −0.8, 95% CI: −1.5; −0.2)), of the lower extremity ($R^2 = .34$ (β −1.7, 95% CI: −2.7; −0.7)) and, consequently, also of the total body ($R^2 = .29$ (β −1.3, 95% CI: −2.1; −0.5)). No significant relation was found between the D4Z4 repeat length and fat infiltration of the upper extremity. Thus, the percentage variance of muscle involvement explained by the regression model was 34% for the lower extremity and 19% for the trunk (Table 7). Overall, compared to men of the same age with a similar D4Z4 repeat length, females had a 10.9% higher percentage of total body muscle involvement.

4. Discussion

To better understand the frequently occurring postural and movement disabilities [9], we aimed to identify the total body pattern of skeletal muscle fat infiltration in a large group of patients with genetically confirmed FSHD. We also studied associations of the degree of muscle involvement with muscle strength and genetic effect size. We demonstrated a pattern of muscle fat infiltration with relatively frequent involvement of the back extensors. In addition, a high percentage of asymmetry was found with more frequent involvement of the right body side. Muscle strength was strongly associated with CT muscle involvement, except for shoulder abduction. Overall, we

Table 2
The percentage of subjects with muscle fat infiltration at either the left or the right body side. Muscles are ordered based on their somatotopic location (from upper to lower extremity) and frequency of involvement.

	n	Percentage of muscles infiltrated with fat
Upper extremity		
m. deltoideus	68	68
m. biceps brachii	70	57
m. triceps brachii	70	43
Forearm flexors/pronators	68	19
Forearm extensors/supinators	68	18
Trunk		
m. trapezius	68	97
m. latissimus dorsi / m. teres major	70	94
m. obliquus ext-int-transv. abdominis	68	90
m. rhomboideus	63	89
m. pectoralis major/minor	70	89
m. serratus anterior	70	89
m. rectus abdominis	69	86
m. longissimus (pars thoracalis)	70	85
m. longissimus (pars lumbalis/iliocostalis)	70	59
m. subscapularis	59	17
m. sternocleidomastoideus	57	14
m. infraspinatus	63	14
m. iliopsoas	69	14
Neck extensors	70	10
Neck flexors(m. longus capitis/m. longus colli)	40	5
Lower extremity		
m. semimembranosus	70	89
m. tibialis anterior	70	71
m. semitendinosus	70	70
m. adductor longus/magnus	70	67
m. biceps femoris	70	67
m. rectus femoris	70	64
m. gluteus minimus	69	61
m. extensor digitorum/hallucis longus	70	60
m. vastus lateralis	70	56
m. gastrocnemius (pars mediale)	70	56
m. peroneus longus/brevis	70	50
m. adductor brevis/m. pectineus	70	47
m. vastus medialis	70	47
m. vastus intermedius	70	44
m. soleus	70	43
m. gracilis	69	42
m. gluteus medius	69	38
m. tensor fasciae latae	70	37
m. sartorius	70	31
m. tibialis posterior	70	31
m. gastrocnemius (pars laterale)	70	31
m. gluteus maximus	69	25

n: Number of patients with a valid right or left muscle CT grade.

found 29% explained variance of muscle involvement by D4Z4 repeat length, corrected for age, which indicates that other than genetic factors must influence disease severity as well.

Based on factor analysis, groups of muscles ('factors') were identified that highly correlated regarding the presence of fat infiltration. Because of the progressive nature of FSHD, we assume that the frequency of involvement of these factors represents the inverse sequence at which the muscles become involved during the course of the disease. We were able to sketch a total body pattern of muscle fat infiltration in FSHD, not taking into account the facial muscles (Fig. 1). The most frequently involved muscle group that was identified ('factor G') consisted of the trapezius, rectus abdominis, and the tibialis anterior with individual frequencies of involvement of 97%, 86% and 71%, respectively. These results support the clinical experience that these muscles are among the earliest affected by FSHD [11,21,22]. The combination of these muscles in the same factor indicates that if one of these muscles is involved, it is likely that the other muscles are also affected, which contrasts with previous studies that described involvement of these muscles in a temporal order [6,10,11,21,22].

Table 3
Sets of muscles determined by factor analysis.

A.	B.	C.	D.	E.	F.	G.
m. iliopsoas	m. gastrocnemius medialis	m. adductor longus/magnus	m. triceps brachii	m. serratus anterior	m. latissimus dorsi	m. trapezius
m. gluteus maximus	m. gastrocnemius lateralis	m. semimembranosus	Forearm flexors/ pronators	m. longissimus lumbalis/iliocostalis	m. longissimus thoracalis	m. rectus abdominis
m. gluteus medius	m. soleus	m. semitendinosus	Forearm extensors/ supinators	m. gluteus minimus	m. rhomboideus*	m. tibialis anterior
m. tensor fascia latae	m. peroneus longus/brevis	m. biceps femoris	m. subscapularis*			
m. sartorius	m. extensor digitorum hallucis longus	m. rectus femoris	m. infraspinatus*			
m. gracilis						
m. vastus intermedius						
m. vastus medialis						

The sets of muscles determined by factor analysis. Within the sets, muscles are categorized by body location in the craniocaudal direction.
* These muscles were omitted from factor analysis because of too few valid data, but are expected to be part of the set of muscles indicated.

	EV	Var (%)	n	Cα	Pi (%)
G	1.5	3.8	3	0.54	99
F	1.8	4.5	2	0.64	94
C	2.5	6.2	5	0.82	91
E	2.1	5.2	3	0.60	90
B	3.3	8.2	5	0.79	76
A	11.1	27.7	8	0.96	74
D	2.2	5.5	3	0.73	51

EV, Eigenvalue of the factor. Var, explained variance of the sets of muscles. n, the number of muscles within the set. Cα, Cronbach's alpha. Pi, percentage of patients with fat infiltration in at least one muscle within the set.

Fig. 2. The Eigenvalue, explained variance, Cronbach's α and the frequency of involvement of the sets of muscles based on factor analysis, organized by frequency of involvement. Colors refer to the body mapping of Fig. 1.

The next group of muscles that could be identified was 'factor F' with a frequency of involvement of 94%. This factor consisted of the latissimus dorsi and the thoracic part of the longissimus muscle, which revealed individual frequencies of involvement of 94% and 84%, respectively. Surprisingly, no study has previously reported such frequent involvement, which may be related to the difficulty encountered when clinically testing these trunk muscles individually. In the present study, a similar testing problem is reflected in the weak association between shoulder abduction and CT grade of the m. deltoideus, which may be caused by the difficulty to exert abduction force when scapula stabilization is poor, even though the m. deltoideus may be unaffected.

The third group ('factor C') was formed by all three hamstring muscles, the rectus femoris, and the adductor longus/magnus with an overall frequency of 91% involvement. Individual frequencies of involvement ranged from 64% to 89%. The high percentage of semimembranosus affliction has previously been reported [3,12], however, the strong relationship between all hamstrings muscles, the rectus femoris and the adductors is a novel finding.

The last group with a high frequency of overall involvement (90%) was 'factor E', which consisted of the serratus anterior (89%), the lumbar part of the longissimus (59%), and the gluteus minimus (61%). The frequent serratus anterior involvement is well known and affects the stabilization of the scapula [11]. The strong relationship with both the lumbar longissimus and gluteus minimus indicates that these muscles are also relatively frequently affected. Again, the involvement of these latter muscles may be overlooked because they are

Table 4
Mean percentage of muscles with CT fat infiltration by body part and by side, and the percentage of asymmetric fat infiltration for all subjects, and for right-handed and the left-handed subjects separately.

	Side				Percentage asymmetric fat infiltration in favor of the right side							
					Handedness							
	Right		Left		All ($n = 70$)		Right ($n = 63$)		Left ($n = 6$)		Left handed – right handed	
	Mean (95% CI)		Mean (95% CI)		Mean (95% CI)		Mean (95% CI)		Mean (95% CI)		Mean (95% CI)	
Total body	50	(45–55)	49	(44–55)	0.5	(−1.2; 2.2)	0.4	(−1.4; 2.3)	0.9	(−5.3; 7.1)	0.5	(−5.8; 6.7)
Upper extremity	36	(29–44)	29	(23–36)	7.0	(2.4; 11.6)	7.4	(2.4; 12.4)	3.3	(−5.2; 12)	−4.0	(−13.1; 5.1)
Trunk	66	(62–71)	67	(63–72)	−1.1	(−3.7; 1.5)	−1.7	(−4.3; 1.0)	4.5	(−8.6; 17.7)	6.2	(−6.9; 19.3)
Lower extremity	45	(38–52)	45	(38–52)	−0.1	(−2.4; 2.2)	0.0	(−2.5; 2.6)	−1.5	(−5.4; 2.4)	−1.5	(−5.8; 2.7)

Table 5
The percentage of subjects with upper extremity muscle fat infiltration at the right, left, both and either side of the body, respectively. Muscles are ordered based on frequency of involvement (either left or right).

	n	Percentage of muscles infiltrated with fat			
		Right (%)	Left (%)	Both (%)	Either (%)
Upper extremity					
m. deltoideus	68	56	56	44	68
m. biceps brachii	70	53	37	33	57
m. triceps brachii	70	37	33	27	43
Forearm flexors/pronators	68	16	12	9	19
Forearm extensors/supinators	68	16	9	7	18

n: Number of patients with a valid right or left muscle CT grade.

Table 6
Average maximum voluntary isometric contraction (MVIC) in kg, during a specific movement, of muscles with normal aspect of muscle tissue (CT grade 1), of muscles with fatty infiltration (CT grades 2–4), and of muscles with different grades of fatty infiltration separately, for both body sides. Spearman correlation coefficients (r^s) between muscle CT grade and MVIC are calculated.

CT muscle aspect	MVIC					
	Upper extremity					
	I: shoulder abduction		II: elbow flexion		III: elbow extension	
	L Mean$^{sd;n}$	R Mean$^{sd;n}$	L Mean$^{sd;n}$	R Mean$^{sd;n}$	L Mean$^{sd;n}$	R Mean$^{sd;n}$
Normal	$9.6^{3.9;30}$	$7.9^{4.0;29}$	$17.3^{6.3;42}$	$21.4^{6.3;31}$	$11.2^{4.1;45}$	$11.2^{3.7;42}$
Fatty infiltration (grades 2–4)	$6.1^{4.3;37}$	$6.3^{4.4;37}$	$12.6^{6.2;26}$	$10.6^{7.8;37}$	$5.9^{4.8;23}$	$5.6^{4.7;26}$
Grade 2	$6.4^{4.4;33}$	$7.1^{5.1;25}$	$15.1^{4.0;16}$	$15.0^{6.6;17}$	$9.6^{3.5;10}$	$8.5^{3.9;12}$
Grade 3	$4.0^{2.8;4}$	$4.6^{1.5;11}$	$12.4^{3.6;6}$	$13.4^{6.6;8}$	$4.7^{4.0;9}$	$5.5^{4.0;9}$
Grade 4	–	$2.9^{-;1}$	$2.3^{0.5;4}$	$2.4^{1.1;12}$	$0.3^{0.7;4}$	$0.0^{0.0;5}$
r^s	−0.41**	−0.27*	−0.66**	−0.75**	−0.64**	−0.68**
CT muscle aspect	Lower extremity					
	IV: knee flexion		V: knee extension		VI: ankle dorsiflexion	
	L Mean$^{sd;n}$	R Mean$^{sd;n}$	L Mean$^{sd;n}$	R Mean$^{sd;n}$	L Mean$^{sd;n}$	R Mean$^{sd;n}$
Normal	$14.6^{6.4;12}$	$16.2^{7.4;17}$	$35.8^{12.5;29}$	$38.9^{14.0;24}$	$18.2^{6.2;25}$	$18.9^{7.5;20}$
Fatty infiltration (grades 2–4)	$9.7^{6.7;56}$	$9.5^{6.9;51}$	$22.7^{11.7;39}$	$22.6^{12.6;44}$	$9.2^{8.1;43}$	$8.2^{7.9;48}$
Grade 2	$15.3^{5.9;18}$	$15.5^{6.2;15}$	$31.0^{7.3;22}$	$31.9^{10.0;22}$	$15.1^{8.1;18}$	$13.9^{7.8;20}$
Grade 3	$11.8^{5.3;12}$	$11.7^{5.9;10}$	$18.3^{10.6;6}$	$21.7^{8.5;11}$	$6.8^{4.5;9}$	$5.7^{4.4;8}$
Grade 4	$5.1^{3.7;26}$	$4.9^{3.9;26}$	$11.5^{7.0;11}$	$9.2^{5.6;11}$	$4.0^{5.0;16}$	$3.8^{5.7;20}$
r^s	−0.67**	−0.66**	−0.71**	−0.75**	−0.68**	−0.68**

The muscles recruited for the different movements were: I: deltoideus, II: biceps brachii, III: triceps brachii, IV: hamstrings, V: quadriceps, VI: tibialis anterior.
* Significant at the level of $p < 0.05$.
** Significant at the level of $p < 0.01$.

Table 7
The regression formula to calculate the estimated muscle fat infiltration from the D4Z4 repeat length, age and gender, for the total body, the upper extremity, the trunk and the lower extremity. A linear regression model was constructed for each body part separately, showing significant contributions of D4Z4 length and age to lower extremity, trunk and total body muscle involvement, and an effect of female gender on total body muscle involvement.

	Intercept	D4Z4 length (kb)		Age (years)		Gender (female)		*R* square (%)
		β	(95% CI)	*β*	(95% CI)	*β*	(95% CI)	
Total body	50	−1.3	(−2.1; −0.5)	0.91	(0.43; 1.38)	10.9	(0.8; 21.0)	29
Upper extremity	53	−0.5	(−1.9; 0.9)	−0.07	(−0.86; 0.73)	12.0	(−5.0; 29.1)	4
Trunk	72	−0.8	(−1.5; −0.2)	0.56	(0.064; 1.06)	7.8	(−0.2; 15.8)	19
Lower extremity	39	−1.7	(−2.7; −0.7)	1.34	(0.76; 1.92)	12.0	(−0.4; 24.4)	34

CI: confidence interval.

harder to test individually. Indeed, manual muscle testing may not be reliable for muscles that act together in moving a joint [3,21].

From individual muscle involvement frequencies (Table 2), it is apparent that the trunk muscles were most frequently affected, with half of the muscles showing fat infiltration in more than 85% of the subjects. The abdominal muscles and the thoracic part of the back extensors showed similar frequencies of involvement (85–90%), which seems to be in contrast with previous studies reporting that the rectus abdominis muscle becomes involved much earlier than the back extensors [21]. Frequent back muscle involvement accompanied by abdominal weakness may be an important determinant underlying postural instability in patients with FSHD [9,23,24]. A remarkable finding was the high percentage of involvement of the oblique and transverse abdominal muscles (90%), next to the rectus abdominis (86%), which indicates that abdominal involvement is widespread.

Interestingly, while all muscles involved in stabilization of the trunk were affected in more than half of the patients, the m. iliopsoas was spared in most patients, suggesting late involvement or perhaps even ‘insensitivity’ of this muscle to fat infiltration by FSHD. This finding is in line with the results of Olsen et al. [3], but seems to be in contrast with those of Wang et al. [12]. However, the latter authors reported that subjects with m. iliopsoas involvement suffered from the infantile form of FSHD, which is characterized by early disease onset, rapid progression, and early wheelchair dependency [25,26]. Unlike trunk instability, dropped head has been documented as an infrequent sign of axial weakness in FSHD [27], which is supported by our finding that neck extensors were involved in only 10% of the subjects.

Remarkably, some muscles were relatively frequently affected, but could not be assigned with other muscles to one specific factor. These muscles are, therefore, not visualized in Fig. 1. One typical muscle was the pectoralis major/minor that was affected in 89% of all patients. This finding is coherent with the fact that loss of pectoralis muscle volume and function has frequently been noted as one of the earliest clinical signs of FSHD [11,21]. Other notable muscles were the m. deltoideus and the m. rhomboideus being affected in 68% and 89% of all patients, respectively. The latter muscle could not be assigned to ‘factor F’ due to a lack of valid data (see Table 3). A relatively high explained variance and Eigenvalue were found for ‘factor A’, which consisted of many of the pelvic and anterior thigh muscles. This finding points toward a relatively strong correlation of involvement, which should not be confused with a high frequency of fat infiltration. In fact, most pelvic and anterior thigh muscles were affected in less than 50% of the patients.

Regarding asymmetry at the level of individual muscles, we found a relatively large proportion (45%) of asymmetric fat infiltration, which is in contrast with three previous imaging studies that reported asymmetric involvement in merely 11% [27] of the whole body, 15% [3] and 18% [12] of the lower extremity, and 27% of upper extremity muscles [12]. This discrepancy may be due to the fact that previous studies evaluated relatively small groups, or focused on a specific subgroup of patients [3,12,27]. In our study, fat infiltration favored the right body side, especially in the upper extremities, which is consistent with previous observations [11,14]. Although it has been postulated that asymmetry of muscle fat infiltration is related to handedness of FSHD patients [11,28,29], we were not able to confirm this hypothesis. We found that left-handed patients showed similar fat infiltration grades for the right upper extremity muscles compared to the left. Additionally, there was no significant difference in asymmetry between right- and left-handed patients. Previously, a study by Tawil et al. [28] also found differences in muscle strength between the left and right body side unrelated to handedness. Overall, the proportion of left-handed patients seems too small to draw definitive conclusions about the cause of asymmetric muscle involvement favoring the right side. Further research is needed to determine whether muscle fat infiltration in FSHD is more severe on the right body side due to genetic, function-related, and/or anatomical/developmental mechanisms.

We found merely a weak association between total amount of fatty infiltration of the body muscles and residual D4Z4 repeat length, corrected for age and gender ($r_s = -0.29$). Previously, inconsistent associations were found between D4Z4 repeat length and muscle strength or clinical severity score [5,6,30,31], while strong associations were found between D4Z4 repeat length and age of onset ($r_s > 0.80$) [7,25,31–33]. One study has found associations between D4Z4 repeat length and fatty infiltration grades of the upper and lower extremities, which were based on a small group ($n = 24$) and not adjusted for age and gender (upper limb: $r_s = 0.55$, $p = 0.007$, lower limb: $r_s = 0.68$, $p < 0.0001$) [12], whereas another study did not find any correlation with lower extremity muscle involvement [3]. We now report significant associations of D4Z4 repeat length with trunk and lower extremity muscles affliction based on total body muscle involvement. Nevertheless, the overall explained variance of muscle involvement by residual D4Z4 repeat length was limited, which implies that there must be other than the known genetic factors that determine the degree of muscle fat infiltration in FSHD. Perhaps the recently identified chromatin modifier of D4Z4, SMCHD1, of which mutations also cause increased expression of DUX4, plays a role in determining the genotype-phenotype association [34,35]. Finally, although penetrance of FSHD is higher in males compared to females [31,36,37], our results counterintuitively reveal that among affected persons the detrimental effect of a certain D4Z4 repeat length on fat infiltration and associated muscle weakness is stronger in females compared to males of the same age with a similar D4Z4 repeat length.

4.1. Study limitations

Although both CT and MRI scans are widely used methods to assess muscle fat infiltration in neuromuscular diseases, MRI is nowadays preferred since it allows objective quantitative assessment of fat infiltration and because patients are not exposed to radiation [1,38,39]. Still, CT is a well standardized method to assess the aspect and shape of muscles allowing operator-independent evaluation of fatty degeneration of superficial as well as deep muscle groups [18,38,40]. A limitation of the present study is the semi-quantitative method that was used to assess muscle fat infiltration. Although patchy replacement of muscle tissue was incorporated in the scale, the fact that the most affected part of the muscle was used for grading may have led to an overestimation of the degree of fat infiltration in muscles that were only locally affected. For example, we found a high percentage of deltoid involvement, while only one patient showed full replacement of deltoid muscle tissue by fat (grade 4) on one body side. Since a CT grade of 2 in the deltoid muscle did not lead to a large decrease in shoulder abduction strength (Table 6), a discrepancy may exist between clinical experience and the frequency of m. deltoideus involvement in the current study. Another limitation is the cross-sectional design, which did not allow to establish temporal relationships. Longitudinal, quantitative muscle imaging studies are required to reliably establish the *sequence* of muscle involvement in FSHD.

5. Conclusion

The current study presents the pattern of fat infiltration based on CT scans of 42 different muscles in 70 patients with facioscapulohumeral muscular dystrophy. Muscle groups were identified based on comparable frequency of involvement using factor analysis. The results indicate that back extensors were relatively frequently affected by FSHD, in addition to the abdominal muscles, which may explain the postural control problems in these patients. Involvement of trunk and leg muscles correlated with the size of the genetic defect, but more so in women than in men. Significant asymmetry of muscle involvement was observed in the upper extremities indicating right-sided predominance, independent of handedness. These novel findings, obtained from a large and relatively unselected study sample, can be used to improve clinical management of patients with FSHD, for instance with regard to restoring postural control and mobility and for prescribing functional exercises.

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