

Review

Muscle imaging in facioscapulohumeral muscular dystrophy research: A scoping review and expert recommendations



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ARTICLE INFO

ABSTRACT

Keywords:

Faciocapulohumeral muscular dystrophy
Muscle imaging
Magnetic resonance imaging
Ultrasound
dual energy X-ray absorptiometry

Clinical trial readiness is an important topic in the field of facioscapulohumeral muscular dystrophy (FSHD). As FSHD is a slowly progressive and clinically heterogeneous disease, imaging biomarkers have been proposed to complement clinical outcome measures. Muscle magnetic resonance imaging (MRI), ultrasound and dual energy X-ray absorptiometry (DEXA) have been used to measure disease severity, activity and progression. We conducted a scoping review of the literature on these imaging modalities to assess gaps in knowledge and subsequently collaborated with a panel of neuromuscular imaging experts to generate recommendations on the road ahead. We systematically searched PubMed, EMBASE and Cochrane Library databases. Three-hundred and twenty-eight studies were screened and one hundred and five studies were included. MRI indices related to intramuscular fat content, STIR positivity and T₂_{water} are used as diagnostic as well as prognostic and monitoring biomarkers. Ultrasound echogenicity can be used as a diagnostic and potentially as a prognostic and monitoring biomarker. DEXA lean muscle mass may be used as an additional monitoring biomarker. Each imaging modality has its own benefits but also challenges. Based on our expert opinions, we propose a roadmap to address these challenges, ensuring the optimal use of each modality in multi-center clinical trials in FSHD.

1. Introduction

Faciocapulohumeral muscular dystrophy (FSHD) is a hereditary muscle disorder and the second most common muscular dystrophy in adulthood, with an estimated prevalence of approximately 12 per 100,000 in the Netherlands [1]. The disease is characterized by slowly progressing muscle weakness over time, resulting in variable functional impairment and, ultimately, wheelchair dependence in approximately 20% of patients [2]. So far, only symptomatic treatment is available. Since the discovery of the genetic mechanism underlying FSHD [3], new therapies are being developed and the first in human clinical trials have been performed (NCT04003974, NCT04004000).

With the development of potential therapeutic approaches, achieving clinical trial readiness is an important item on the research

agenda [4–6]. A critical facet of clinical trial readiness is the development of suitable clinical outcome measures (COMs) and biomarkers [7]. The current criteria applied by regulatory agencies state that the effect of any potential therapy should be clinically relevant; therefore, COMs are most often used as primary endpoints. However, COMs have several limitations: their results can depend on patient cooperation and learning effects; in slowly progressive disorders like FSHD they frequently show a low sensitivity to change in the typical clinical trial timeframe of one to two years; and often little is known about their validity and reliability [8]. This may result in trials needing an exceptionally long follow-up period, a large number of participants, or failing to demonstrate disease progression and treatment effects [9]. These setbacks may be avoided by adding biomarkers, which measure an indicator of a pathogenic process or response to an intervention, to COMs in clinical trial

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protocols [8].

In FSHD research, the non-invasive assessment of disease severity, activity, progression, and treatment effects obtained from muscle imaging is essential [10]. In early imaging studies computed tomography (CT) was explored [11], but it has been replaced by other techniques. Currently, the most commonly used techniques are muscle magnetic resonance imaging (MRI), muscle ultrasound and dual energy X-ray absorptiometry (DEXA). Muscle MRI can quantify fat replacement as well as edema [10] and has been studied extensively, providing biomarkers for multiple purposes, such as diagnosing and monitoring disease. Muscle ultrasound can quantify fatty replacement and assess the presence of fibrosis [12]. It has been used both as a diagnostic and a monitoring technique in neuromuscular disorders, but it is relatively novel in FSHD research. It is particularly suitable for studies in children because it is relatively fast and easy to use. DEXA quantifies tissue mass instead of fat replacement or fibrosis and has been used to monitor disease in FSHD [13].

Considering that MRI, ultrasound and DEXA measure different aspects of disease severity or progression, and few studies have compared them head-to-head, the choice of a particular technique for clinical trial design may be difficult and needs to be considered in light of the available knowledge.

In this context, the 265th European Neuromuscular centre (ENMC) international workshop was organized, during which the latest muscle MRI and ultrasound results in FSHD research were presented and the role of both techniques in future clinical trials was discussed [5]. To expand on this workshop's report, we propose a scoping review of the current knowledge of the relevant muscle imaging methods used in FSHD. The following research questions were formulated:

1. What is currently known on the most relevant imaging techniques used in FSHD research?
2. How are these imaging techniques used in FSHD studies and what evidence supports that role?
3. Which novel imaging applications have been used in FSHD research?

We aim to answer these questions and subsequently provide the interpretation of our findings from a panel of neuromuscular imaging experts, generating their view on the challenges ahead and the strategy to ensure the optimal use of each imaging biomarker studied in clinical trials in FSHD.

2. Methods

To answer our research questions on muscle imaging in FSHD, a systematic literature search was performed, which is described in the next section. The results from this search were reported in this review in accordance with the PRISMA Extension for Scoping Reviews (PRISMA-ScR) guideline [14]. This guideline describes a systematic approach to map the literature on a topic, identifying main concepts, theories and knowledge gaps. To describe the challenges ahead and propose a strategy on how to move forward in the field, we synthesized our findings with expert opinions.

2.1. Eligibility criteria

We included only studies targeting FSHD and studies regarding skeletal muscle imaging, in particular the imaging techniques that were considered most relevant for FSHD research: muscle MRI, muscle magnetic resonance spectroscopy (MRS), muscle ultrasound and DEXA.

2.2. Exclusion criteria

Exclusion criteria included: 1) Studies on imaging other than skeletal muscle imaging (e.g. cardiac ultrasound), or skeletal muscle imaging outcome not reported, or studies on other techniques than imaging (e.g.

genetic testing); 2) Studies on other neuromuscular disorders, or FSHD cases not separately described; 3) Case reports; 4) Trial registrations, reviews, meeting- and workshop reports, conference abstracts of the latter two; 5) Studies published in languages other than English.

2.3. Search strategy and study selection

Article selection was performed by two authors (S.V. and S.T.), following the Radboud University Medical Library Guidelines for performing a Literature search [15]. We performed a search in PubMed and Cochrane databases on the 12th of November 2023 to find relevant studies. We combined the MeSH term 'dystrophy, facioscapulohumeral muscular' (Search 1) or the term 'facioscapulohumeral muscular dystrophy' (Search 2), with the following terms included in Title or Abstract: 1. For MRI: 'MRI' OR 'magnetic resonance imaging'; 2. For MRS: 'MRS', 'magnetic resonance spectroscopy', 'magnetic resonance spectroscopic imaging' OR 'MR spectroscopic imaging'; 3. For DEXA: 'dual energy x-ray absorptiometry' OR 'DEXA'; 4. For ultrasound: 'ultrasound' OR 'ultrasonography'. We performed additional EMBASE searches using the same search terms to add potential gray literature, such as conference abstracts, to our review. These searches only generated a limited number of new relevant studies from last year. These were all studies that were recently presented at international conferences but were not yet submitted or accepted for publication. We therefore decided to limit our MRI EMBASE search to results from the last two years (2022 and 2023). After consulting with a medical librarian, we added another extensive search, including additional search terms and combinations, performed on the 12th of November 2023 (See Supplementary materials for a detailed list of terms). Abstracts were screened for inclusion and relevance according to our research questions stated in the introduction, and studies were selected based on full texts.

3. Results

Three hundred and twenty-eight studies were identified through searching the databases and were screened for relevance (See Supplementary materials). Two hundred and twenty-three studies were excluded since they did not fulfill the inclusion criteria. The remaining one hundred and five studies were included in this review and their main findings are used to answer our research questions. The search strategies and resulting studies can be found in the Supplementary Materials. Considering the large number of studies encountered, their results are described separately for each imaging modality in different subsections.

3.1. MRI and MRS biomarkers in FSHD

3.1.1. Diagnostic MR biomarkers in FSHD

The diagnosis of FSHD – especially in clinically typical and familial cases – does not necessarily require the use of muscle imaging and can be confirmed through genetic testing [6]. However, muscle imaging can help guide the diagnosis of FSHD in patients with a suspected yet unclassified neuromuscular disorder. This group of patients may include FSHD patients without a positive family history for FSHD or patients with an atypical FSHD phenotype [5,16], though more evidence is needed to support the use of muscle MRI in the latter group. Most studies focus on imaging of fat replacement in muscles of FSHD patients, either using qualitative imaging with T1-weighted (T1w) sequence or quantitative imaging with a Dixon sequence (Fig. 1). Several FSHD cohort studies described the muscle MRI patterns of involvement in FSHD [17–31]. One large lower limb and truncal muscle T1-weighted (T1w) MRI cohort study found that in 67% of all participants the following pattern was present: signal abnormalities in at least one hamstring muscle (semimembranosus, semitendinosus or biceps femoris muscle) and at least one abdominal muscle (obliquus and transversus or rectus abdominis muscle), combined with bilateral iliopsoas sparing [32]. Another study reported on upper extremity and shoulder girdle MRI,

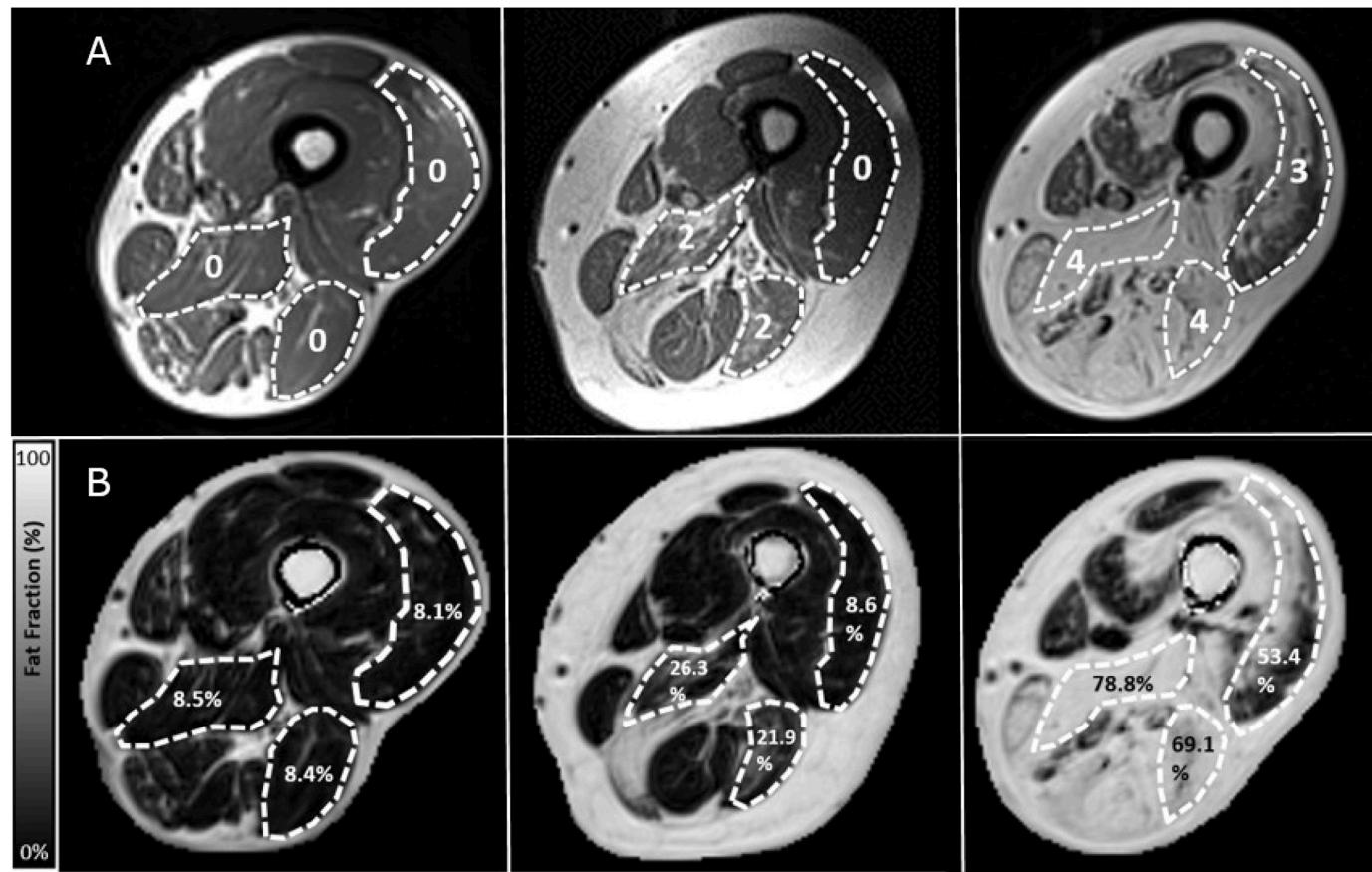


Fig. 1. T1-weighted and Dixon images of the upper legs of three different FSHD patients.

A. MRI T1-weighted images of the upper legs of FSHD patients with the semi-quantitative modified Lamminen score displayed in the vastus lateralis, adductor magnus, and biceps femoris long head (the three delineated muscles). Grade 0: normal, Grade 1: Mild with only traces of increased signal intensity, Grade 2: Moderate with increased signal in less than 50% of affected muscle, Grade 3: Severe with increased signal intensity in more than 50% of affected muscle and Grade 4: Entire muscle replaced by abnormal signal. The semimembranosus muscle of the middle patient has a Lamminen grade 1. B. Corresponding Dixon fat-fraction maps with the quantified average fat fraction in percent displayed in the same delineated muscles. Fat fraction ranges from 0% (a muscle with no fatty replacement; black) up to 100% (a muscle that is fully fatty replaced; white).

where a combined involvement of muscle abnormalities on T1w images of at least one trapezius, one latissimus dorsi, one pectoralis major, one serratus anterior and a complete sparing of subscapularis, supraspinatus and infraspinatus muscles was present in 66% of the patients [33]. A recent study defined combinations of involvement and sparing of specific upper and lower extremity muscles able to distinguish FSHD from overlapping disorders with high accuracy, sensitivity and specificity just based on the MR images and even combinations that are pathognomonic for FSHD [16].

Besides the pattern of muscle involvement in FSHD, two other important characteristics of muscle abnormalities have been reported: 1. Asymmetric involvement of muscles, which was found in at least one muscle pair, in both upper and lower extremity muscles and in the majority of the patients [17,18,33,32,34]; 2. A proximal-distal gradient in muscle fat replacement. MRI studies of the lower extremities have described this gradient in quantitative fat fraction, usually with high fat fractions at the distal muscle end that decrease towards the proximal end. This points to the distal end as the primary disease initiation site in leg muscles [35,36].

Most studies have been conducted with only FSHD1 patients or just a small number of FSHD2 patients within a larger cohort. Recently, a study in a large FSHD2 population ($n = 34$) showed that the radiological pattern is similar to that of FSHD1, except that in FSHD2 there was a predominant involvement of the lower limbs [37].

3.1.2. Monitoring and prognostic MR biomarkers in FSHD

Multiple cohort studies have shown that MRI fat fraction correlates strongly with commonly used COMs (see studies in Table 1). Furthermore, multiple studies have found increases in fat fraction in a study period of one year, proving its sensitivity to change (see studies in Table 2) and its utility as a monitoring biomarker. Since this sensitivity to change is robust enough to detect progression in fat replacement in a clinical trial time span, MRI may be attractive for measuring response in future clinical trials (Fig. 2). Several trials already have used muscle MRI as secondary endpoint [38–40]. Of note, the reported fat fraction changes over time, though robust, vary between studies, and seem to depend on the method used, the included patients, the selected muscles, the baseline fat fraction, and the location and number of slices analyzed [41], amongst others. These factors influencing fat fraction changes should be considered when fat fraction is used as a biomarker in clinical trials. In contrast to the strong cross-sectional correlations between COMs and MRI fat fraction, correlations between changes in COMs and changes in fat fraction over time are weak to moderate [41]. This is not surprising, given the fact that many COMs are considerably less sensitive to change in FSHD than MRI fat fraction, and therefore their use in trials is limited [42].

MRI outcomes also correlate with histopathological findings and DUX4 gene expression. DUX4 is a transcription factor which causes FSHD by its abnormal expression in skeletal muscles. Several studies have shown the predictive value of signal hyperintensities on a short-tau

Table 1

Cross-sectional correlations between muscle MRI and clinical outcome measures.

Study	Analysis method	Patients	Correlation
Tasca et al. [32] *	Semi-quantitative	269	Lower limb T1-MRI, Upper girdle T1 MRI and Global T1-MRI scores correlated with Ricci score (CC = 0.934, 0.767 and 0.927, respectively). Higher Ricci score (odds ratio = 1.9, 95% CI = 1.5–2.6, $p < 0.001$) increased the risk of having 1 STIR+ muscle on lower limb MRI.
Gerevini et al. [19]	Semi-quantitative	30	Fat replacement scores and muscle atrophy scores correlated with clinical severity in the FSHD group and non-FSHD group.
Regula et al. [20]	Semi-quantitative	20	Mean MRI score correlated with mean MMT scores (CC= – 0.89). Several separate muscle MMT scores and corresponding MRI scores correlated significantly, especially in the shoulder girdle and legs.
Tasca et al. [33] *	Semi-quantitative	108	T1-MRI score correlated with Ricci score (CC = 0.76).
Leung et al. [21]	Semi-quantitative	47	Significant correlations between the mean fat infiltration score and quantitative muscle strength in the hamstrings ($r=-0.56$), the quadriceps with MRI scores greater than 2 ($r=-0.69$) and 10-meter walk time. In additional dataset: significant relationship between the mean fat infiltration and quantitative muscle strength in the biceps brachii ($r=-0.71$), in the triceps ($r=-0.56$), in the hamstrings with MRI scores >2 ($r=-0.80$) and in the quadriceps with MRI scores >2 ($r=-0.56$).
Olsen et al. [22]	Semi-quantitative	18	Mean MRI score of the corresponding muscles correlated with the MMT scores of hip abduction, hip adduction, hip extension, knee flexion, knee extension and ankle dorsal flexion (CC 0.54 - 0.82).
Giacomucci et al. [37]	Semi-quantitative	34	Reduced lower limb-T1w-MRI score and total-T1w-MRI score correlated with Ricci score (CC= 0.580 and 0.524, respectively).
Woodcock et al. [43]	Semi-quantitative	11 (children)	Amount of intramuscular fat and muscle atrophy correlated strongly with the Ricci score, FSHD clinical score, 6MWT, MFM and PUL.
Frisullo et al. [44]	Semi-quantitative	25	T1 MRI score correlated strongly with the Ricci score (CC = 0.83).
Iosa et al. [45]	Semi-quantitative	12	Total MRI score correlated strongly with total MMT score (CC=–0.81).
Wang et al. [46]	Quantitative	36	Sigmoidal relationship between fat replacement and quantitative tibialis anterior strength.
Mul et al. [17]	Quantitative	140	Mean fat fraction correlated with MFM, 6-MWT, Ricci score and FSHD clinical score (CC 0.701 - 0.845). Separate MMT scores correlated with fat fraction of the separate muscle groups, except for the plantar flexors.
Fatehi et al. [47]	Quantitative	35	Total mean pixel intensity correlated with the MMT scores of both thighs, the MMT sum score, the Ricci score and MFM D1, D2 and MFM total score (CC –0.37 - 0.68).
Andersen et al. [48]	Quantitative	45	Composite fat fractions correlated significantly with FSHD clinical score, 5TSTST, 14SST and 6MWT (CC= 0.62 - 0.79). Quantitative strength measurements correlated with corresponding fat fraction of muscle groups (CC= –0.33 - –0.74).
Mellion et al. [49]	Quantitative	17	MFF and MFI variables of different muscle groups correlated with TUG (CC 0.71 and 0.83), FSHD-TUG (CC 0.73 and 0.73) and RWS (left CC –0.71 and –0.53, right CC –0.61 and –0.65).
Kan et al. [35] \$	Quantitative	7	Tibialis anterior MRC scores correlated significantly with muscle fraction.
Dahlqvist et al. [50]	Quantitative	10	Mean muscle fat fraction correlated with FSHD clinical score (CC = 0.92), knee strength (CC = 0.88), 6-MWT (CC = 0.87, stair climb test (CC = 0.85) and 5TSTST (CC = 0.88).
Lassche et al. [51]	Quantitative	12	MRI contractile cross-sectional area correlated strongly with maximum voluntary contractile strength.
Lareau-Trudel et al. [18]	Both	35	Mean fat fraction correlated with Ricci score and inversely correlated with manual muscle testing score and MFM sub score D1.
Janssen et al. [24] \$	Both	41	Quantitative fat fraction of lower limb correlates with Ricci score ($R^2 = 0.90$). Muscle fraction * muscle area correlated with quantitative muscle strength for the quadriceps and hamstring ($R^2 = 0.57$).
Gerhalter et al. [52]	Both	19	The MMT score of plantar flexion was related to the mean fat fraction of the gastrocnemius and soleus muscle (CC=–0.52) and the MMT score of dorsal flexion was related to the fat fraction of the tibialis anterior muscle (CC=–0.79).
Hamel et al. [53]	Both	20	MMT sum scores correlated strongly with semiquantitative T1 score (CC=–0.81) and muscle fat fraction (CC=–0.77) of the lower extremity.
Banerji et al. [54]	Both	26	Lower limb fat fraction correlated strongly with three clinical severity scores: the Ricci score (CC = 0.61), FSHD clinical score (CC = 0.60) and MMT sum score (CC= –0.63).

5TSTST=5 Times Sit To Stand Test, 6-MWT= 6-minute walking test, 14SST= 14 Step Stair Test, CC= correlation coefficient, FSHD-TUG= adapted FSHD Timed Up and Go, MFM= Motor Function Measure, MMT= manual muscle testing, PUL= Performance of the Upper Limb, RWS= Reachable Work Space, TUG = Timed Up and Go.

* and \$ indicate known overlapping study populations.

inversion recovery (STIR) sequences in identifying muscles with signs of active muscle damage, including fibrosis, muscle fiber necrosis and inflammation on histopathology and increased levels of DUX4 signature gene expression [59–61]. This was confirmed in a one-year follow-up study by Wong et al. [62] and in a more recent study of a new cohort by the same authors using whole muscle MRI [63]. Another study showed that PAX7 target gene repression, also a proposed FSHD biomarker, was associated with MRI fat replacement [46].

Muscle fat fraction changes over time also depend on their fat fraction at baseline [24,36,41,58,64]. Muscles with a low (specific numbers vary between studies, ranging from 0.1 to 0.4) or high (ranging from 0.6 to 0.8) fat fraction at baseline showed a smaller increase of fat fraction over time than muscles with an intermediate fat fraction at baseline. These findings are similar to longitudinal FF results of other (slowly) progressive muscular dystrophies [65,66]. Taking this into account, another measure of fat replacement was proposed: the 'muscle fat infiltration' (MFI), which is the fat fraction calculated within voxels with

less than 50% fat [67]. The MFI was used as secondary monitoring/-response biomarker in the phase 3 losmapimod randomized controlled trial (NCT05397470), though it has not yet been investigated in or validated by other studies. STIR positive muscles also showed a higher increase in fat fraction compared to STIR negative muscles [41,50,64], as did muscles with elevated T2_{water} relaxation times [68].

In conclusion, an intermediate MRI fat fraction at baseline and T2_{water} STIR hyperintensity/T2_{water} relaxation time all seem to be associated with faster disease progression in FSHD. This can be of use in a clinical context, but also shows that these measurements can be used as prognostic biomarkers.

3.1.3. MR spectroscopy in FSHD

Several small cohort studies have addressed the pathophysiological aspects of skeletal muscle in FSHD using mrs techniques. Studies employing phosphorus MR spectroscopy (³¹P-MRS) found abnormal energy metabolism in fat-replaced muscles, as opposed to the muscles

Table 2

Longitudinal muscle MRI studies: change over time and correlations between progression in MRI outcomes and progression in clinical outcome measures.

Study	Number of Patients	Follow-up period	Change in MRI outcomes over time	Correlation with clinical outcome measures
Janssen et al. [55]	13	12 weeks	Mean progression of fat fraction was 6.7% (95% CI 4.3%–9.1%) per year. In all muscles fat fraction increased, except in the gracilis, sartorius and vastus lateralis muscles. The largest progression was found in the adductor magnus, with 19% per year (95% CI 12%–26%). Seven new TIRM hyperintensities were identified at follow up.	n/a
Janssen et al. [24]	41 at baseline, 11 at FU	4 months	Muscle fat fraction showed an average increase of 0.054 ± 0.12 per year. In muscles with a baseline fat fraction of > 0.25 and < 0.75 the increase in fat fraction was higher (0.18 ± 0.15 per year) than in more and less infiltrated muscles (0.00 ± 0.10 and 0.043 ± 0.10 , respectively).	n/a
Wang et al. [46]	36 at baseline, 35 at FU	12 months	Median change in fat fraction was 0.01 [IQR –0.01, 0.03] (mean \pm SD: 0.02 ± 0.07), with the greatest change in the semimembranosus muscle. 63% of muscles with a fat fraction of 0.40–0.50 at baseline showed a fat fraction increase of ≥ 0.10 , while no muscles with a fat fraction higher than 0.80 had a fat fraction increase of ≥ 0.10 . A higher STIR rating was associated with higher fat fraction change.	Muscle Fat Fraction Burden Index increase was associated with a decrease in 6MWT and an increase in Go 30' (both $p < 0.001$).
Ferguson et al. [56]	16 at baseline, 15 at FU	12 months	In 7 muscles progression of T1w observed fat replacement was seen, of which 4 muscles showed STIR + signal at baseline.	n/a
Monforte et al. [57]	100	12 months	Mean T1 score was 71.9 ± 50.79 at baseline and 72.28 ± 50.92 at follow up ($p < 0.001$). 97.3% of the muscles did not show changes at follow-up; an increase of at least one point was found in 0.53% of the muscles. No significant difference was found in the number of STIR+ muscles between baseline and follow-up.	n/a
Andersen et al. * [48]	45 at baseline, 40 at FU	436.3 ± 43 days	Absolute fat fractions progressed in all muscles, except the lumbar paraspinal muscles, and progression slowed when muscles reached a fat fraction of 0.60. The largest increase was seen in the thigh muscles. The composite absolute muscle fat fraction increased 0.036 ($p < 0.001$), after excluding outliers it progressed with a yearly increase of 0.022 ($p < 0.001$).	Changes in muscle fat fraction did not correlate with progression in FSHD clinical score and muscle strength.
Dahlqvist et al. [58]	43	436.3 ± 43 days	All muscles showed an increase in fat fraction of 2.89% annually ($p < 0.0001$). This was higher in STIR+ muscles than in STIR-muscles, 5.0 \pm 4.0% vs. 2.3 \pm 3.3% ($p < 0.0001$). The severity of STIR hyperintensity at baseline correlated weakly with the increase of fat fraction in STIR+ muscles ($R = 0.39$, $p = 0.001$). Muscles with fat fractions of 40–60% at baseline showed a greater increase in fat fraction than muscles with lower or higher initial fat fractions ($p < 0.0001$) and had a higher percentage of STIR+ muscles ($p < 0.05$).	n/a
Fatehi et al. [47]	35, 11 third visit	12.5 months time point 1 - 2, 13.5 months time point 2-3	Total mean pixel intensity increased significantly between timepoint 1 and 2 ($p = 0.000$), with a rate of 0.62/year (0.01 – 1.79). Patients with three timepoints also showed a significant increase in total mean pixel intensity.	Changes in total mean pixel intensity did not correlate with change in clinical scores.
Dahlqvist et al. [50]	10	32 months	Mean muscle fat fraction increased significantly between first and last visit (0.43 ± 0.28 to 0.46 ± 0.27 , $p < 0.0001$). The muscles used in the T2 _{water} analysis had a fat fraction increase of $3.2 \pm 4.1\%$ ($p < 0.0001$), but their T2 _{water} did not change ($p = 0.43$). A higher T2 _{water} at baseline predicted a higher rate of fat replacement in muscle ($R = 0.30$, $p < 0.0001$).	Changes in muscle fat fraction did not correlate with change in clinical scores ($p > 0.50$).
Heskamp et al. [36]	9 at baseline, 7 at FU	3 years and 8 months	Lower extremity muscle fat fraction showed an average increase of $4.7 \pm 5.9\%$ ($p = 0.043$) during the FU period, which is a yearly increase of $1.3 \pm 1.6\%$. Fat fraction progression varied, depending on baseline fat fraction. Muscles with a fat fraction of 30–40% at baseline showed the largest increase.	n/a

6-MWT= 6-minute walking test, FU= follow up. Descriptive statistics reported as median [interquartile range] or mean \pm SD.

* indicate known overlapping study populations.

that appeared normal on MRI ($n = 9$ and $n = 41$) [24,69]. A cohort study ($n = 36$) using multi-voxel ¹H-MRS showed metabolic abnormalities (a reduced trimethylamine/creatinine ratio) in FSHD muscles, even when no fatty replacement was visible [70]. These studies indicate that some MRS techniques – like ¹H-MRS – may be able to detect early muscle pathology in FSHD prior to fat replacement and therefore they might be suitable for use as prognostic biomarkers. However, the validity, generalizability, and sensitivity to change of each new metric still needs to be established. Detailed discussion of the challenges and future opportunities of these metrics is beyond the scope of this review.

3.1.4. Segmentation techniques and new MRI applications in FSHD

As manual segmentation of muscles is cumbersome and time consuming, the adequate and efficient use of quantitative MRI fat fraction as a biomarker in multicenter trials would benefit from (semi-) automated segmentation methods [5]. Two of the first studies that used automated segmentation of muscle tissue on T1w and T2w images to calculate the intramuscular fat fraction in FSHD reported that manual correction could not be avoided with this technique, particularly for patients with severely fat replaced muscles [18,71]. Chambers et al. proposed an automatic segmentation method on T1w images using a live-wire technique, that did not require manual correction [72]. A semi-automated quantitative segmentation method enabled a successful

whole-muscle analysis in a longitudinal study of leg muscles in FSHD patients [36]. In several studies including healthy individuals and patients with muscular dystrophies, whole-muscle automated segmentation was successfully performed [73–75]. Recently, an automatic segmentation technique for quantitative analysis of muscle FF and MFI in separate muscles in FSHD was validated and implemented on different MRI systems [67]. Of note, this technique is proprietary and is currently being used in clinical trials of losmapimod in FSHD (NCT05397470). However, for definitive use in future clinical trials, their results should be confirmed in natural history studies. The latest development in automatic muscle segmentation in quantitative MRI is the use of deep learning models. These have been assessed in small cohorts of subjects without neuromuscular disease with excellent accuracy and reproducibility in calculating FF [76–79]. However, for implementation in clinical trials in FSHD, validation of these techniques in neuromuscular diseases is needed.

Artificial intelligence has also been used to identify patterns of muscle involvement to diagnose FSHD [16], to distinguish between myositis and FSHD patients based on MRI results [80], and to predict muscle T_{2water} relaxation times and muscle fat fractions using texture analysis [81] or radiomics and machine learning [82], which might obviate the need for quantitative MRI sequences. Furthermore, artificial intelligence is being used to develop an online tool for diagnosing neuromuscular disorders using MRI (MYOGUIDE) [83].

The use of dynamic MRI with neuromuscular electrical stimulation was explored in one cohort study ($n = 34$), which showed that this technique could potentially provide, by quantitatively assessing changes in muscle deformation (strain), additional information to monitor disease progression [84]. Further studies implementing this method are needed to validate these findings.

3.2. Dual-energy X-ray absorptiometry (DEXA)

3.2.1. Monitoring biomarker use in FSHD

In contrast to the large body of literature available on muscle MRI in FSHD research, DEXA has only been used in few small studies. It is primarily used clinically to measure bone mineral density, but it has also been used to evaluate body composition in FSHD [85,86]. As a tool for investigating muscle mass, DEXA was first used in three clinical trials: 1) a pilot trial using prednisolone ($n = 8$), that did not show significant changes in lean mass [87]; 2) a pilot trial using diltiazem ($n = 19$), which also showed no changes in lean mass [88], and 3) a larger randomized controlled trial in albuterol ($n = 84$), which only showed a significant increase in lean mass in the high dose group compared to placebo [89]. Later DEXA was also assessed in several small cross-sectional cohort studies ($n = 11$ to 19), showing that FSHD patients often had increased regional fat tissue mass with decreased regional lean tissue mass and that quantitative strength correlated strongly with lean tissue mass ($\rho = 0.8\text{--}0.9$, $p < 0.001$) [90–93]. A recent large, multi-center longitudinal study aiming to improve clinical trials readiness in FSHD (the ReSolve study; $n = 185$) has also used DEXA to quantify lean muscle mass [13]. Their first baseline results have recently been published, showing moderate correlations between upper- and lower extremity lean tissue mass and corresponding quantitative strength (ρ varying between 0.5 and 0.7), and corresponding functional outcome (ρ varying between 0.3 and 0.6) [94]. The first longitudinal results showed a median change in whole body lean muscle mass of 0.8% over 1 year [95].

3.3. Muscle ultrasound

3.3.1. Diagnostic and monitoring ultrasound biomarkers in FSHD

Muscle ultrasound has been assessed in several cohort studies in FSHD, with both cross-sectional and longitudinal designs [96–101]. The

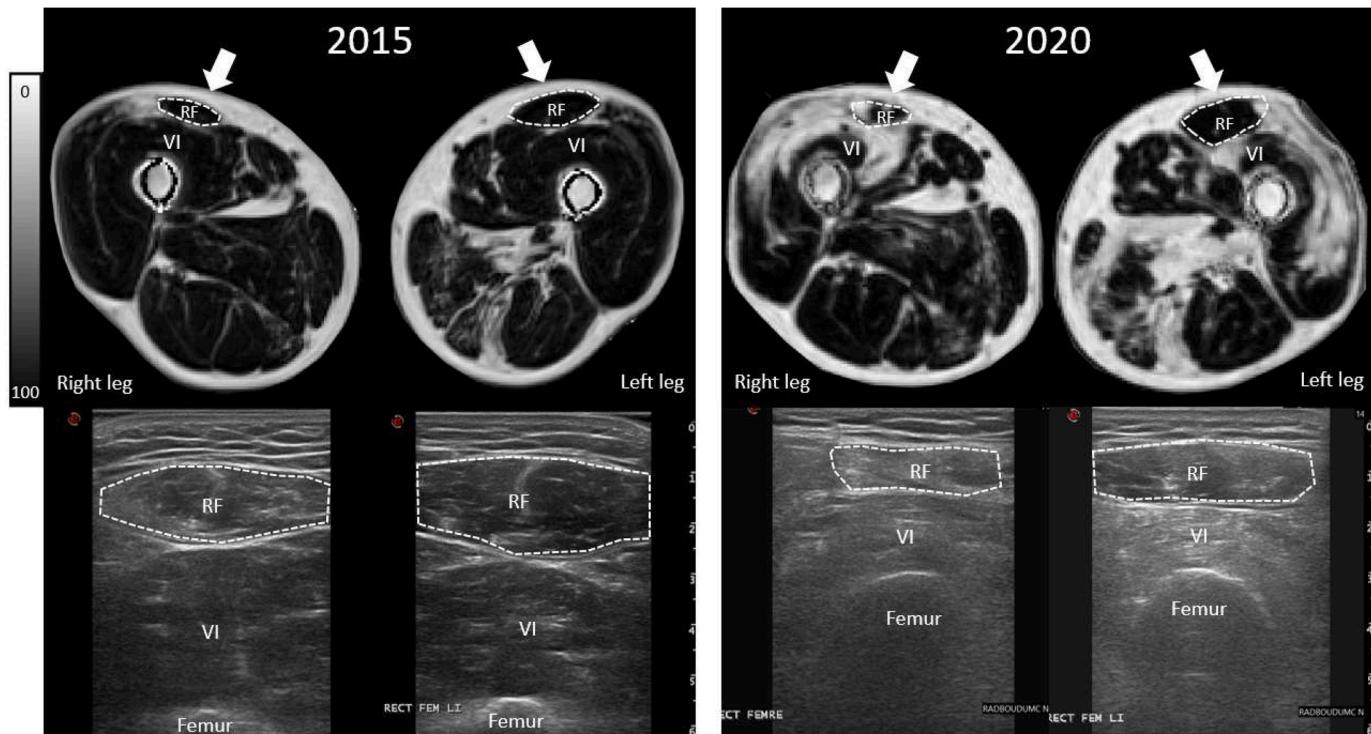


Fig. 2. Longitudinal muscle imaging of thigh muscles of a FSHD patient.

MRI Dixon fat-fraction maps of both thighs and muscle ultrasound images of the anterior thigh muscles of both legs in one FSHD patient with a follow-up period of five years. RF = rectus femoris. VI = vastus intermedius. In 2015, the right RF had a FF of 13.6%, an echogenicity z-score of 1.52 and Heckmatt score of 1; while the left RF had a FF of 5.1%, an echogenicity z-score of -0.99 and a Heckmatt score of 1. In 2020, the right RF had a FF of 23.8%, an echogenicity z-score of 3.5 and a Heckmatt of 3, while the left RF had a FF of 6.3%, an echogenicity z-score of 1.10 and a Heckmatt score of 2.

relationship between muscle ultrasound and COMs in FSHD was assessed by four studies: two cross-sectional ($n = 27$ and $n = 115$, respectively) and two longitudinal cohort studies ($n = 20$ and $n = 22$, respectively) compared both semi-quantitative and quantitative muscle ultrasound results with an extensive clinical examination. They found strong correlations between FSHD clinical severity and functional scores and the mean echogenicity z-score (ranging from 0.6 to 0.8; $p < 0.001$) and between FSHD clinical severity and functional scores and the mean Heckmatt score (ranging from 0.73 to 0.8; $p < 0.001$) [96,99,100,102]. These findings show that semi-quantitative and quantitative muscle ultrasound can be used as biomarkers for disease severity in FSHD (Fig. 3). Both longitudinal studies also assessed the sensitivity to change of muscle ultrasound, laying the foundation for the use of muscle ultrasound as a monitoring biomarker [96,99]. They found that single muscle echogenicity z-scores and Heckmatt scores as well as sum-echogenicity z-scores and Heckmatt scores increased over a period of one or two years, though results were not always significant on a single muscle level and the majority of participants were children [96]. However, considering the patients in these cohorts did not show changes in COMs over the observation period, these results may suggest that changes in ultrasound abnormalities precede clinical deterioration in FSHD.

3.3.2. Muscle ultrasound and muscle MRI in FSHD

The first study to compare muscle MRI with ultrasound was a cross-sectional cohort study that included five FSHD patients and compared results of quantitative muscle ultrasound with a location-matched quantitative muscle MRI of the leg muscles. They reported strong correlations between echogenicity z-scores and muscle fat fraction and T1w signal intensity and showed that quantitative muscle ultrasound had a larger dynamic range than quantitative MRI [97]. Another cross-sectional cohort study of twenty-seven patients compared the results of quantitative ultrasound analysis to the results of quantitative muscle MRI of leg muscles [100]. They showed that MRI fat fraction correlated strongly with ultrasound echogenicity z-score (Spearman $\rho = 0.9$, $p < 0.05$). The discrepancies between quantitative muscle ultrasound and muscle MRI results were also evaluated; they were found both in the early and late-disease stages. Muscle ultrasound detected early changes in muscle architecture that were not visible on MR images, possibly caused by changes such as fibrosis. Muscle MRI was better at detecting late stages of fatty replacement: some muscles that were fully fatty replaced on MR images had abnormal textures on ultrasound and thus abnormal Heckmatt scores, but a pseudo-normal echogenicity (i.e. that of fat) (Fig. 4). It was also noted that MRI is better equipped to assess muscle edema, as muscle ultrasound images may show signs of muscle edema, but there is no adequate ultrasound equivalent for STIR positivity. The most recent comparison of quantitative muscle ultrasound and semi-quantitative MRI ($n = 13$) showed higher echointensity in muscles from FSHD patients compared to controls and a higher median echointensity in muscles with more fat replacement according to the Mercuri scale [103]. However, no echogenicity reference values were used in this study and no discrepancies encountered between muscle ultrasound and MRI were described in detail. Preliminary results of a small cohort study comparing muscle ultrasound and MRI longitudinally suggest that muscle ultrasound is most suited as imaging technique in early disease stages and MRI in late disease stage [104] (Fig. 2). This means that the role of muscle ultrasound in current clinical trials that mainly include intermediate stage patients is probably limited, though it has been used as monitoring biomarker in one clinical trial together with MRI, in which echogenicity showed strong correlations with MFI [105]. Muscle ultrasound may be more useful in future trials that want to target patients in earlier disease stages to prevent irreversible muscle damage.

3.3.3. New muscle ultrasound techniques in FSHD

A new quantitative muscle ultrasound method that creates three-dimensional (3D) muscle images was introduced to visualize muscle volumes instead of cross-sections, which is useful in the assessment of heterogeneously affected muscles [106]. One study ($n = 31$) reported that quantitative parameters other than muscle echogenicity – such as the standard deviation of echo intensity and residual attenuation – show strong correlations to muscle weakness and may have biomarker potential [106]. While muscle ultrasound mainly uses static imaging, dynamic ultrasound techniques that capture muscle movement have also been piloted in FSHD. One strain-elastography pilot-study combined dynamic ultrasound with speckle tracking to quantify muscle deformation. They were able to evaluate the effects of muscle pathology in relation to contraction, strength and movement generation. However, as it was a small study that included only four patients, further research will be needed to establish any biomarker potential [107]. Finally, artificial intelligence may facilitate a more consistent use of quantitative muscle ultrasound, but it has not yet been used extensively in neuromuscular disorders such as FSHD [5,108,109].

4. Box 1: expert opinions

4.1. Methods

Accompanying the scoping review, we wanted to provide expert recommendations on the optimal use of muscle MRI, ultrasound, and DEXA as imaging biomarkers in clinical trials in FSHD. To develop a diverse expert panel we identified FSHD experts from the clinical, ultrasound and MRI/MRS fields from within our Dutch network of neuromuscular centers (authors KM, LH, AH, DC, HK, NV, NV and BvE; from the Radboudumc, LUMC and UMCU), added the organizer of the recent ENMC workshop on muscle imaging in FSHD [5] (GT) and one of the US FSHD imaging experts (DL). After the first two authors performed the scoping review (as described in Section 2), they discussed the reviews results and together they formed the first interpretation of these results. This resulted in the first draft of the manuscript, which they presented to the expert panel. The manuscript was iterated upon in seven rounds of edits, allowing the panel to share, discuss and edit their opinions, while the first authors served as mediators in the process. All authors ultimately agreed on the expert opinions here presented.

4.2. Results

Building from the current body of literature on imaging in FSHD, we will address the gaps in knowledge and challenges ahead, before suggesting a strategy ensuring appropriate use of muscle MRI, muscle ultrasound and DEXA as imaging biomarkers in upcoming FSHD trials.

4.2.1. Muscle MRI

Quantitative muscle MRI fat fraction is already widely used as a secondary outcome measure (monitoring biomarker) in clinical trials in FSHD as well as a prognostic biomarker. Nonetheless, it would be helpful to know more about the long-term longitudinal MRI data. Ideally, this data should be obtained with a follow-up period of at least 5 years (but preferably longer, up to 10 years) and three or more visits with equal time periods. Such long-term longitudinal data, in which fat fraction is acquired in combination with COMs, can be used to create a prediction model of fat fraction development and its association with clinical outcome. If fat fraction is reasonably likely to predict clinically relevant changes in clinical outcome in the long-term, fat fraction can be used as surrogate endpoint. One way to achieve this is to conduct large prospective cohort studies combining MRI and clinical data; another is to meta-analyze MRI and clinical data from earlier natural history

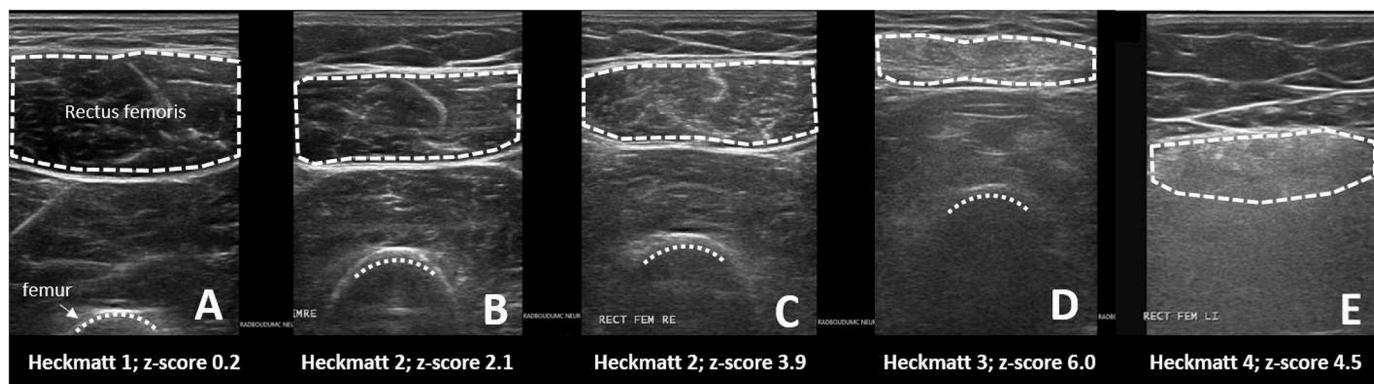


Fig. 3. Ultrasound images of the right rectus femoris of FSHD patients and their Heckmatt and echogenicity z-scores.

A = Appearance of a normal muscle; B/C = Appearance of a muscle with an increased muscle grayscale level with a distinct bone echo; D = This muscle shows a markedly increased grayscale level with diminished bone echo; E = This muscle shows very strongly increased grayscale level with a total loss of bone echo. The important pitfall of decreasing echogenicity z-scores in severely affected muscles can be observed here.

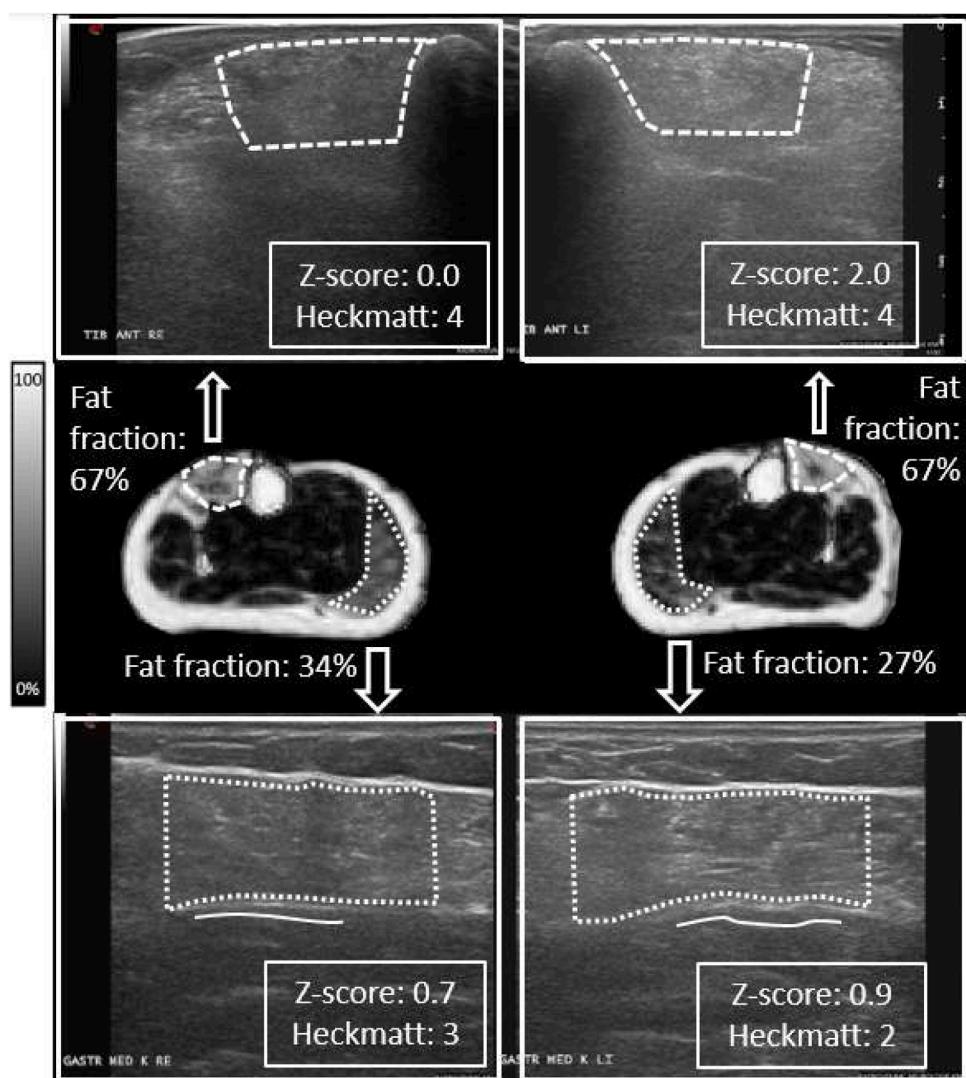


Fig. 4. Heckmatt and z-score echogenicity discrepancy versus MRI Dixon fat fraction in lower leg muscles of an FSHD patient.

Bilateral fatty replacement of the tibialis anterior and medial head of the gastrocnemius muscle in an FSHD patient, as seen on MRI Dixon fat-fraction maps and muscle ultrasound images, with corresponding fat fractions, echogenicity z-scores, and Heckmatt scores. This example clearly shows the discrepancies between the Heckmatt and echogenicity z-scores. NB: z-score <2 is normal. Heckmatt 1 is normal.

studies and placebo data from clinical trials. This approach could also be used to derive exact yearly increases in fat fraction per muscle or distribution of fat fraction changes over time.

STIR positivity and T₂_{water} relaxation time are also established prognostic biomarkers that can be used in clinical trials, while their use as monitoring biomarkers has been less exploited, mainly for technical reasons (i.e., it is difficult to implement a protocol assessing muscle T₂_{water} relaxation time with wide coverage). Similarly to fat fraction, there is a knowledge gap concerning long-term longitudinal data, and the relationship between change in STIR positivity or T₂_{water} relaxation time and change in clinical outcome still needs to be established [68, 110]. T₂_{water} relaxation time is preferred because it is a quantitative measurement, but to use it appropriately one needs to take into account known influencing factors, such as physical exercise [111] and fat fraction [110,112], which influences overall T₂ relaxation time.

Another important challenge for the use of muscle MRI parameters as endpoints in multicenter clinical trials, is the current inter-site variability of the applied MRI protocols and segmentation methods. Ideally, all sites should use the same acquisition protocols, with stringent quality control of the data with rapid turnaround time and centralized data analysis. Fat fractions should also be analyzed for whole muscles or a number of slices evenly distributed over the whole muscle length, using at least 5 slices [36], to account for the proximal-distal gradient. To overcome the limitations mentioned above related to the outstanding need of reliably using T₂_{water} relaxation time as a monitoring biomarker alongside fat fraction, the attendees of the recent ENMC workshop on muscle imaging in FSHD discussed the possibility of developing a comprehensive protocol with whole-body coverage, quantitative fat and water analysis and patient friendly scan time [5]. Whole-body water-fat separation imaging can be done in approximately 30 min, whereas whole-body T₂ mapping takes considerably longer. In the latter case, a complete T₂ mapping protocol could be replaced by a more anatomically-selective protocol (in which at least the shoulder girdle, and lower extremities are included) or could be applied with lower resolution. Segmentation of muscles or muscle groups should be done centrally either manually or using a (semi-) automated approach, though these still need to be validated in larger cohorts.

In conclusion, MRI fat fraction can be used as a diagnostic (if analyzed in terms of distribution in 'key' muscles, such as the trapezius, subscapularis, supra- and infraspinatus, iliopsoas, rectus femoris and hamstring muscles), prognostic and monitoring biomarker, though additional studies are needed to establish its use as a surrogate endpoint and its optimal use in multi-center clinical trials. Similarly, MRI STIR positivity and T₂_{water} can be used as prognostic (and monitoring) biomarkers in clinical trials, but for their use as surrogate endpoints additional data are required.

4.2.2. DEXA

There are no historic or longitudinal data yet to support the use of DEXA as a monitoring or prognostic biomarker in clinical trials. Important differences between DEXA and muscle MRI or ultrasound are that DEXA is unable to evaluate individual muscles and that it is unable to evaluate muscle fatty replacement or edema; it can only measure overall muscle mass. It remains unclear whether DEXA is sensitive to change and how changes in muscle mass relate to clinical outcome longitudinally; therefore, sufficiently large longitudinal cohort studies combining DEXA results with clinical outcomes are needed. While we await the results of the ReSolve study [95], the use of DEXA in clinical trials is probably limited and best reserved as a potential additional monitoring biomarker.

4.2.3. Muscle ultrasound

To establish quantitative muscle ultrasound derived measures as a monitoring or prognostic biomarker in clinical trials, more knowledge is needed about its longitudinal relation to COMs and its sensitivity to change, that should be validated in a sufficiently large longitudinal

cohort study. Future studies should also determine how muscles with conflicting semi-quantitative and quantitative muscle ultrasound results progress over time and how these muscles relate to MRI findings, in order to evaluate each technique's optimal use [100]. To create the optimal ultrasound protocol, studies should assess which muscles are the most efficiently and reliably evaluated in FSHD.

The steps required in adequately implementing quantitative muscle ultrasound for use in multicenter studies depend on those future studies' design. For cross-sectional studies, each center should collect muscle- and equipment-specific reference values. For longitudinal studies, however, reference values are not needed, because the difference in echogenicity from the baseline value over time can be used for analysis of changes in muscle health. Training is required for centers to adequately acquire data, focused on probe location, pressure and angulation to ensure low inter-rater variability, while data processing and analysis can be confined to one center that is equipped with a team of physicians experienced in muscle ultrasound assessment [12]. In conclusion, muscle ultrasound appears suited for use as a diagnostic biomarker in FSHD. Considering the limited amount of available data on muscle ultrasound natural history and its sensitivity to change, additional studies are needed to establish the use of muscle ultrasound derived measurements as monitoring or prognostic biomarker.

4.2.4. Limitations

The same challenges hampering optimal use of imaging methods in FSHD research led to similar challenges for this review: the studies included here used different designs and protocols, which hindered reliable comparison of their results. Also, most MRI studies had relatively large sample sizes, whereas MRS, DEXA and ultrasound studies were often smaller, limiting the data's generalizability. Finally, a larger body of literature was available for MRI, as opposed to the relatively scarce literature on DEXA and ultrasound, which made our conclusions on the latter modalities less solid.

5. Conclusion

After discussing the results from our extensive review of the available literature on muscle MRI, muscle ultrasound and DEXA in FSHD, our expert opinion is that: 1) muscle MRI indices related to intramuscular fat content can be used as diagnostic, prognostic and monitoring biomarkers; 2) MRI STIR positivity and T₂_{water} can be used as prognostic and monitoring biomarkers; 3) muscle ultrasound can be used as a diagnostic, and potentially prognostic and monitoring biomarker; 4) additional studies are needed on all imaging techniques to address gaps in knowledge. Finally, each imaging technique still has its own challenges to navigate, which should be addressed in trial and other study design. Here we presented expert recommendations on how to navigate these challenges and proposed a roadmap to ultimately validate them as surrogate endpoints in multi-center clinical trials in FSHD.

Ethics approval and consent

Ethical approval was not required because of the study nature (no humans or animals included).

Funding

This project has received funding from the charitable foundation Prinses Beatrix Spierfonds, Grant number W.OR12-22 and W.OR18-07, and the FSHD Stichting, grant number WP35 and WP36.

CRediT authorship contribution statement

Sanne C.C. Vincenten: Writing – original draft, Methodology, Investigation, Data curation, Conceptualization. **Sjan Teeselink:** Writing – original draft, Methodology, Investigation, Data curation,

Conceptualization. **Karlien Mul:** Writing – review & editing, Methodology, Conceptualization. **Linda Heskamp:** Writing – review & editing. **Hermien E. Kan:** Writing – review & editing. **Arend Heerschap:** Writing – review & editing. **Donnie Cameron:** Writing – review & editing. **Giorgio Tasca:** Writing – review & editing. **Doris G. Leung:** Writing – review & editing. **Nicol C. Voermans:** Writing – review & editing, Methodology, Conceptualization. **Baziel G.M. van Engelen:** Writing – review & editing, Methodology, Conceptualization. **Nens van Alfen:** Writing – review & editing, Methodology, Conceptualization.

Declaration of competing interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests:

KM is a consultant for Avidity Biosciences.

LH was a consultant for AMRA medical in 2021 and 2022.

HK reports Research support from Philips Healthcare and Trial Support from ImagingDMD. Reimbursements were received by the Leiden University Medical Center.

GT has acted as consultant on advisory boards and participates as steering committee member on a study in FSHD with F. Hoffmann-La Roche.

DL reports research support for her institution from Friends of FSH Research, F. Hoffmann-La Roche AG, Fulcrum Therapeutics, Seattle Children's Research Institute, and the University of Kansas Medical Center Research Institute.

NV is the chair of the FSHD European Trial Network (unpaid) and is a member of the steering committee of Fulcrum Phase 3 trial (REACH), for which she has a financial arrangement with the Radboudumc.

BvE reports grants from Global FSH, Stichting Spieren voor Spieren, Prinses Beatrix Spierfonds and Dutch FSHD Foundation. **BvE** is a consultant for Fulcrum, Avidity, Dyne, Arrowhead, Biomarin and Facio (all fees payed to the institution). **BvE** has a patent EP20120740236 with royalties paid to Euroimmun.

NvA performs editorial duties for Wiley Publishing Inc. and is an ultrasound instructor for Sonoskills, for which all payments go to their employer.

Acknowledgements

Several authors of this publication are members of the Radboudumc Center of Expertise for neuromuscular disorders (Radboud-NMD), Netherlands Neuromuscular Center (NL-NMD) and the European Reference Network for rare neuromuscular diseases (EURO—NMD).

Supplementary materials

Supplementary material associated with this article can be found, in the online version, at [doi:10.1016/j.nmd.2025.105274](https://doi.org/10.1016/j.nmd.2025.105274).

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