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Personalized antioxidant supplementation improves muscle strength, physical activity, and quality of life in patients with FSHD1: A real-world longitudinal study

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Abstract

Facioscapulohumeral muscular dystrophy (FSHD) is an autosomal dominant myopathy characterized by progressive muscle weakness. Oxidative stress plays a central role in its pathogenesis. This longitudinal, real-world study evaluated the effect of personalized antioxidant supplementation on quadriceps muscle strength (maximum voluntary contraction; MVC_Q), physical activity, and quality of life (QoL) in patients with FSHD1. Patients (aged 15-76) at Montpellier Hospital received tailored antioxidant supplementation (vitamins C and E, zinc, copper, and selenomethionine) based on blood analyses during annual visits: 107 completed one year and 74 continued for two more years of supplementation. Linear mixed model analyses showed a significant annual increase in the MVC of both dominant and non-dominant quadriceps muscles (+0.83 kg/year, +1.25 kg/year, respectively), improvement in physical activity, especially sports, and in QoL domains, notably physical role. Sex and age influenced outcomes: supplementation benefits were more pronounced in men and younger patients. Supplementation withdrawal resulted in the benefit loss, while attrition was unrelated to disease severity or treatment response. Overall, our findings suggest that personalized antioxidant supplementation may slow FSHD progression, improve muscle strength and quality of life. Baseline muscle strength and clinical scores, including the Brooke upper extremity scale score, allowed stratifying patients in responders and non-responders. These parameters underscore the importance of baseline assessments to predict outcomes and highlight the need of controlled studies to confirm these preliminary results.

1. Introduction

Facioscapulohumeral muscular dystrophy (FSHD) is one of the most prevalent autosomal dominant muscular dystrophies, with an estimated prevalence between 1 in 8000 and 1 in 20 000 individuals in Europe and between 1 in 16 000 and 1 in 38 000 people in the United States of America [1,2]. FSHD is primarily characterized by progressive muscle weakness, especially in facial and shoulder muscles. Genetically, FSHD is associated with deletions on chromosome 4q35, specifically within the D4Z4 repeat array [3], which characterizes Type 1 FSHD. Type 2 FSHD involves mutations in chromatin modifiers, such as SMCHD1 [3]. These genetic alterations result in DNA hypomethylation and toxic overexpression of the double-homeodomain transcription factor DUX4 [3], leading to muscle differentiation defects, atrophy and oxidative stress [4–7]. Currently, there is no cure for FSHD, and treatments remain largely symptomatic and multidisciplinary. Management involves regular assessments tailored to the individual patient needs, focusing on assistive devices, pain relief, and monitoring of respiratory or non-muscular complications. In patients with FSHD, aerobic exercise and muscle strength training [3,8,9] improve the overall physical fitness without exacerbating fatigue or muscle damage. Various pharmaceutical strategies (e.g. prednisone, beta-2 agonists, myostatin inhibitors, anti-inflammatory and antioxidant agents) also show potential positive effects [10], although their long-term benefits remain unclear.

Evidences suggest that oxidative stress plays a pivotal role in FSHD pathology [11–18]. This hypothesis is supported by studies in FSHD muscle biopsies showing deregulated expression of enzymes associated with oxidative stress and increased oxidative damage [13,19]. Moreover, *ex vivo* experiments showed that primary myoblasts from patients with FSHD exhibit increased sensitivity to exogenous pro-oxidants [15, 18]. In addition, several genes involved in oxidative stress susceptibility are affected by DUX4 derepression, suggesting a complex interplay between oxidative stress and genetic factors in FSHD [20]. Interestingly, DUX4 overexpression in mouse C2C12 myoblasts results in acute cellular toxicity, which can be alleviated by the addition of antioxidants, such as ascorbic acid and vitamin E, to the culture medium [7]. However, in FSHD myocytes, oxidative stress activates a DNA damage response signaling pathway, which in turn leads to increased DUX4 expression [21], highlighting a complex interplay between oxidative stress and genetic factors in this disease.

In a previous study, we observed that reduced physical performance in patients with FSHD correlates with significant redox imbalance and oxidative stress [13]. Specifically, these patients exhibit elevated levels of systemic oxidative stress markers, such as lipid peroxides and oxidized DNA, and decreased concentrations of vitamin C and essential trace elements, particularly selenium, a glutathione peroxidase cofactor, and zinc, which is crucial for superoxide dismutase activity. Notably, the ratio of reduced (GSH) to oxidized glutathione (GSSG) is strongly decreased in most patients as a consequence of GSSG accumulation. GSH can directly interact with reactive oxygen species, but its main function is as a substrate for glutathione peroxidase, which detoxifies lipid peroxides. Typically, GSH levels decline during oxidative stress; however, this is not observed in patients with FSHD, suggesting a reduced efficiency of GSH-dependent enzymes. Additionally, the low vitamin C to ascorbyl radical ratios in patients with FSHD suggest that GSH is not effectively recycled to regenerate

vitamin C, highlighting a potential impairment in antioxidant recycling mechanisms. Based on these findings, a randomized placebo-controlled trial (n = 53 patients with FSHD) evaluated a targeted antioxidant strategy to address FSHD-specific oxidative stress [14]. The supplementation included vitamin C (500 mg) and vitamin E as alpha tocopherol (400 mg), selenium as selenomethionine (200 µg), and gluconate of zinc (30 mg), chosen for their crucial roles in antioxidant pathways and their potential to reduce oxidative damage [22–24]. The results showed significant improvements in quadriceps muscle strength (primary outcome), enhanced antioxidant defenses and a marked reduction in oxidative stress markers (secondary outcomes) [14]. Moreover, magnetic resonance imaging highlighted improved muscle quality and decreased fat content in patients receiving daily antioxidant supplementation [17]. Interestingly, patients with the lowest baseline copper/zinc ratios and lipid peroxide levels exhibited the most significant increases in dominant quadriceps strength [14]. This suggests that these oxidative stress markers may predict the response to antioxidant supplementation. Conversely, copper levels and copper/zinc ratios tended to be higher in non-responders, highlighting the importance of maintaining a proper balance between copper and zinc to optimize the effects of antioxidant supplementation [14]. In addition, vitamin E concentration was negatively correlated with the levels of GSSG and oxidized DNA [14], supporting the hypothesis that vitamin E plays a crucial antioxidant role in mitigating oxidative damage at the cellular level. However, such correlations were not observed for vitamin C, indicating the need of a balanced intake of both vitamins. Although, vitamin E and copper were within reference ranges, our previous study emphasized the importance of maintaining adequate levels of these antioxidants in patients with FSHD. Indeed, the positive correlations between lipid peroxides and both vitamin C/vitamin E and copper/zinc ratios [14] suggested their involvement in oxidative stress regulation. Notably, the positive correlation between vitamin C and lipid peroxides at the end of treatment may reflect a pro-oxidant effect of vitamin C [25]. The increase in glutathione peroxidase activity at the end of treatment [14] further suggested that the selenium levels were sufficient to support enzyme activity [26]. Overall, these findings imply that an optimal balance among vitamin C, vitamin E, zinc, copper, and selenium may be necessary for effective protection against oxidative stress in patients with FSHD. They also underscore the importance of nutrition in managing muscle health, particularly in neuromuscular disorders. Our analysis of dietary intake in 152 patients with FSHD routinely followed revealed no significant correlation between dietary intake and plasma concentrations of these nutrients in both sexes [27], highlighting that a balanced diet alone might be insufficient, and targeted supplementation could be needed to address nutritional gaps. Additionally, caution is needed with high-dose antioxidant supplementation to prevent toxicity and nutrient imbalance [28].

Based on these previous findings, we developed a personalized antioxidant supplementation strategy that take into account the variability in oxidative stress levels among patients with FSHD in order to optimize the therapeutic outcomes. This personalized approach involves tailoring the dose of vitamins and trace elements according to the plasma concentrations of specific biomarkers using a decision-tree model integrated into our routine clinical practice. This model is currently under patent pending status (FR/3140199 and WO2024/ 062051) and the exclusive license has been transferred to the KONDREE company. We collected real-world data to assess the effect of such personalized approach on muscle strength, physical activity, and health-related quality of life (QoL) in patients with FSHD. We focused particularly on quadriceps strength, chosen for its relevance to the specific pathological changes that may reflect accurately FSHD progression [13, 14,17,29].

2. Materials and methods

2.1. Study design and ethics

The “PERsonalised medicine and SPECialized TherapY for better liVing with FSHD” (PERSPECTYV FSHD) is an open-label, longitudinal real-life study (NCT02622438) conducted within the framework of the usual routine care at the Clinical physiology department of Montpellier University Hospital, France, from April 2015 to July 2024. This study was approved by the Montpellier University Hospital ethics committee (ref. 14-02-06sc; ID-RB: 2014-A00153-44) and adhered to the 1964 Declaration of Helsinki and European guidelines for good clinical practice. This project, submitted in 2014, was classified as “research aimed at evaluating routine care” and the Montpellier University Hospital (promoter of the study) requested to obtain the participants' nonopposition following their informed consent. Therefore, informed written consent for participation in the study and for data publication was obtained from participants or their parents/guardians. Eligible patients were aged >7 years and had a molecular and clinical diagnosis of FSHD. Exclusion criteria were: pregnant women, inability to understand the nature and goals of the study and/or communication difficulties with the investigator, major protected by law (guardianship, curatorship or under judicial protection).

2.2. Participants and procedure

Patients older than 15 years with a molecular (D4Z4 repeat genotyping) and clinical diagnosis of FSHD, were referred for neurological consultations. In France, the molecular diagnosis of FSHD is done at two specialized reference centers. For this study, blood samples were sent to the Molecular Genetics Laboratory, Department of Medical Genetics, Timone Children's Hospital in Marseille, for genetics analysis. The genetic diagnosis is based on the D4Z4 repeats size measurement. Specifically, genomic DNA is first digested using the EcoR1 enzyme, followed by detection of D4Z4 fragments by Southern blotting with a probe (P13 e11) that hybridizes to the region immediately upstream of the first repeat. In patients with FSHD1, the fragment sizes range from 10 to 38 kb (vs 40 to 350 kb in control individuals). As D4Z4 repeats are also found on chromosome 10, an additional enzymatic digestion step the Bln1 enzyme (specific for repeats on chromosome 10) allows the effective distinction between the two sources of D4Z4 repeats.

The trial involved an initial three-year monitoring period: initial assessment (visit 0, V0), tailored one-year antioxidant supplementation with assessment at the end of year 1 (V1), and a subsequent two-year follow-up with assessments at the end of year 2 (V2) and 3 (V3). Some participants extended their follow-up and antioxidant supplementation to four (V4) and five years (V5). Each annual visit (V0 to V5) included a comprehensive clinical and laboratory assessment to identify potential morbidities, such as cardiovascular and respiratory disorders, insulin resistance, diabetes, hyperthyroidism, iron-related diseases, hypercholesterolemia, hyperglycemia, renal function abnormalities. All patients underwent a clinical examination that included a medical history and the collection of the Brooke Scale (upper extremities) [30] and Vignos Scale (lower extremities) [31] scores. Evaluations comprised manual testing of

the shoulder external rotators, elbow flexors, knee extensors, foot dorsiflexors, and plantar flexors. The FSHD clinical score (CS; range 0–15; higher scores indicate more severe involvement of facial, upper limb, leg, scapular, pelvic girdle, and abdominal muscles) was recorded. Visual inspection of the spine was performed to assess the presence of scoliosis or lumbar hyperlordosis, classified as normal, mild, or severe. The 10-grade Clinical Severity Score (CSS), developed by Ricci et al. (1999) [32], was used to assess disease progression based on weakness in various regions, from the face and shoulders to pelvic and lower limb muscles. Higher scores indicate greater involvement of pelvic and proximal lower limb muscles, reflecting advanced disease. To account for age differences at examination, an age-corrected CSS was calculated using the formula proposed by van Overveld et al. (2005) [33]: $\text{age-corrected CSS}^{**} = ((\text{CSS} \times 2) / \text{age at examination}) \times 1000$. Multi plying by two converts scores into whole numbers, and multiplying by 1000 facilitates the interpretation of results. Routine blood analyses were performed at each visit. Functional muscle testing, assessments of physical activity (Voorrips questionnaire) [34] and health-related QoL (SF-36 questionnaire) [35] were performed at each visit. The doses of vitamins and trace elements were adjusted using the plasma concentrations of vitamin C, vitamin E, zinc, selenium, and copper and a decision-tree model (patent pending). The maximum permitted daily doses were 500 mg vitamin C, 400 mg DL-alpha tocopheryl-acetate (vitamin E derivative), 45 mg zinc gluconate, 0.15 mg copper, and 200 µg selenomethionine. Participant adherence to the antioxidant supplementation was documented at each follow-up visit. Data were collected in individualized electronic medical records, which were curated for analysis.

2.3. Endpoints

The primary endpoint was the change in maximal voluntary contraction of quadriceps muscles (MVCQ) from baseline, assessed as previously described [13, 14, 17]. Muscle strength was evaluated every year by assessing the MVCQ (in kg) using an adapted exercise bench (Kettler, Germany) as previously described [13, 14, 16, 17]. Participants were seated with knees and hips flexed at 90°, and a strain gauge was connected to a computer interface (Biopac, Acknowledge, France). Three to five maximal trials were performed to ensure at least two values with less than 300 g variability. The best value was designated as the MVCQ. The quadriceps with the highest MVCQ was considered as the dominant quadriceps muscle (MVCQD), and the other as the non-dominant quadriceps muscle (MVCQND).

Secondary endpoints focused on physical activity and health-related QoL changes. Physical activity was evaluated with the Voorrips Questionnaire, which considers household, sports and leisure activities [34]. Patients were asked to describe their activity type, number of hours per week, and period of the year in which the activity was normally performed. All activities were classified according to posture and movement. Each activity is scored, and the sum of the three scores provides a total score; higher scores indicate increased physical activity. QoL was measured with the SF-36 questionnaire that evaluates physical and mental health domain [35]. Each domain is composed of four areas: physical functioning, role limitations due to physical health problems, and bodily pain compose the physical domain; general health perceptions, vitality (energy/fatigue), social functioning, role limitations due to personal or emotional problems, and mental health (psychological distress and well-being) compose the mental health domain. In each area, scores range from 0 to 100 [35]. The mean physical (PCS) and mental (MCS) component summary scores were calculated for each domain [36]. Higher SF-36 physical and mental component scores (PCS and MCS, respectively) indicate better physical and psychological well-being, respectively.

2.4. Statistical analysis

Data are presented as means and standard deviations (\pm SD). Normality was assessed using the Shapiro-Wilk test. The sample size calculation was based on a prior trial [14]. To detect a clinically relevant improvement of 0.5 kg in MVCQD with a standard deviation of 2, a correlation coefficient of 0.90, an alpha of 0.05, and a power of 0.90, a sample size of 36 was required. To accommodate an estimated 10% dropout, the sample was increased to 40. Changes over time were assessed using linear mixed-effects models that take into account individual variability to estimate the overall effects of time (V0, V1, V2, V3) and covariates (e.g. sex, age) on muscle strength (MVCQ) and other outcomes. Given the absence of validated minimal clinically important differences for quadriceps strength in patients with FSHD, two distribution-based thresholds were used to interpret the clinical significance of observed muscle strength changes. These thresholds included: (1) X1, defined as $0.5 \times$ standard deviation (SD) of the change scores (ΔX), and (2) the minimal detectable change at 95% confidence (MDC95), calculated as $1.96 \times$ standard error of measurement (SEM). The discriminatory capacity of each threshold was evaluated using receiver operating characteristic (ROC) curves and the area under the curve (AUC) and 95% confidence intervals. The optimal cutoff for differentiating responders and non-responders was identified using the Youden index. Sensitivity and specificity at this optimal threshold were reported. Additionally, subgroup analyses categorized patients according to the sex assigned at birth, age (younger vs older than 50) and baseline MVCQD levels (low: ≤ 10 kg, medium: 10–20 kg, high: ≥ 20 kg). The models included fixed effects for time, groups and their interaction (time* groups), to assess differential treatment effects over time. This interaction models heterogeneous effect of treatment over time across the studied groups. Random effects included subject-specific intercepts to account for baseline differences, as well as random slopes to capture individual variability in change across annual visits (V0, V1, V2, V3). Fixed effects comprised time, sex, age and MVC at baseline to evaluate their influence on outcomes. Multiple comparisons were controlled using the Benjamini-Hochberg false discovery rate (FDR) procedure, with adjusted p values. To assess potential bias from dropouts, the baseline characteristics were compared between patients who continued the tailored antioxidant supplementation (V3, V4, V5) and patients who dropped out after V1, V3 or V4 using contingency tables and Chi-square tests of independence to examine associations with disease severity (Vignos scale) or treatment response (based on prior MVCQD improvements). In the QoL analysis, outliers were identified using the interquartile range method (threshold: 1.5 times the interquartile range) and were removed as necessary ($n = 4$). No data imputation for missing information was done because it could introduce bias given the FSHD progression variability. Effect size for group comparisons between visits were quantified using the Cohen's d, categorized as small if $0.20 \leq d < 0.50$, medium if $0.50 \leq d < 0.80$, and large if $d \geq 0.8$. The Spearman's rank correlation coefficient was used to assess associations between variables. All analyses were performed with R, RStudio [37] (version 4.5.1, <http://www.r-project.org>) and SigmaPlot version 14; significance was set at $p < 0.05$.

3. Results

3.1. Participants

Initially, 152 patients with FSHD1 were enrolled, but 45 were excluded for reasons detailed in Fig. 1. Consequently, 107 patients (59 men, 48 women) completed the assessment at year 1 (V1). Among them, 74 patients (44 men, 30 women) continued the tailored supplementation and completed V2 and V3. After V3, 39 patients were lost to follow-up or stopped the antioxidant supplementation, mainly due to the COVID-19 pandemic ($n = 34$) and personal reasons ($n = 5$). Due to restructuring and reorientation within the clinical department, routine care was discontinued, and only 35 (V4) and 20 patients (V5) could be monitored. In addition, 18 patients went to only one follow-up visit after the COVID-19 pandemic. Throughout the study, no adverse effect was reported, in line with the existing literature [38,39].

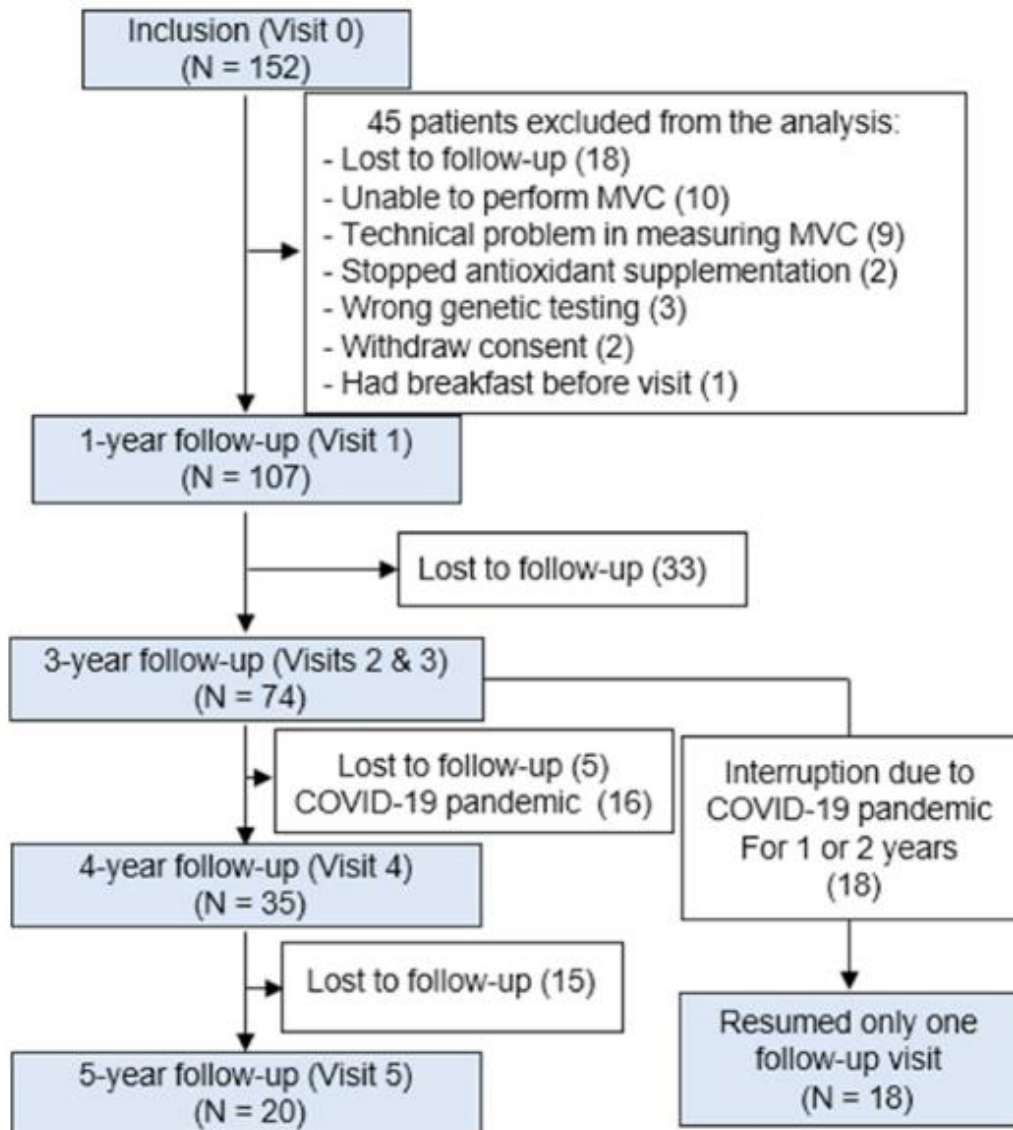


Fig. 1. Study flowchart.

3.2. Demographic and clinical characteristics

The baseline demographic and clinical characteristics of the 107 patients are detailed in [Table 1](#). All patients travelled to the Clinical Physiology Department, University Hospital in Montpellier, France for this routine care: 98 came from France (including 26 from Occitanie region), 8 from other European countries, and 1 from the United Kingdom. Nineteen patients were smokers during the entire study period and 10 patients had type 2 diabetes. No demographic, clinical, or treatment-related factors led to exclusion.

Table 1

Baseline demographic and clinical characteristics of patients with FSHD.

At baseline (visit 0, V0), demographic data and genotype results (D4Z4 repeat number) were recorded. Leg function was evaluated using the Vignos functional scale that ranges from 1 (ability to walk and climb stairs without assistance) to 10 (bed-bound). No patient had a score = 10. The Brooke functional scale ranges from 1 (ability to abduct the arms in a full circle until they touch above the head) to 6 (no function of hands). No patient had a score ≥ 5 . Abbreviations: BMI, body mass index; FSHD, facioscapulohumeral muscular dystrophy; max, maximum; min, minimum; n, number of subjects; SD, standard deviation.

Demographics	All patients with FSHD (N = 107)	
	Mean (SD)	Median (min–max)
Age, years	47.0 (16.1)	48 (15–74)
≤18 years, n (%)	7 (6.5)	
BMI, kg/m ²	23.7 (4.5)	22.7 (14–39.1)
Sex assigned at birth,		
Men, n (%)	59 (55.1)	
Age mean (SD)	48.2 (15.6)	51 (16–74)
BMI, mean (SD)	23.5 (3.6)	22.8 (15.4–33.8)
≤18 years, n (%)	2 (3.4)	
Women, n (%)	48 (44.9)	
Age, mean (SD)	45.7 (16.7)	46 (15–70)
BMI, mean (SD)	23.8 (5.4)	22.5 (14–39.1)
≤18 years, n (%)	5 (10.4)	

Genotyping	
D4Z4 repeat number, mean (SD)	5.9 (2.1)
Men	6.1 (2.2)
Women	5.7 (1.9)
Arm function	
Brooke score (n, %)	
1 and 2	19 (17.8)
3	78 (72.9)
4	10 (9.3)
Leg function	
Vignos score (n, %)	
1	49 (45.8)
2	28 (26.2)
3	8 (7.5)
≥4	22 (20.6)
FSHD-CS (0–15), median (Q1–Q3)	5 (4–7.75)
0, n (%)	0 (0)
1–2, n (%)	13 (12.1)
3–7, n (%)	67 (62.6)
8–15, n (%)	27 (25.2)
FSHD-CSS (0–10), median (Q1–Q3)	3 (1 – 4)

The mean number of D4Z4 repeats was 5.9. At baseline (V0), 48.6% of patients were older than 50 years and 20.6% and 9.3% of patients had a Vignos scale score (leg function) ≥ 4 and Brooke score (arm function) of 4, respectively, indicating moderate lower and upper limb impairment. In agreement, the median CS was 5 (IQR: 4–7.75), indicating mild to moderate disease severity. The median CSS was 3 (IQR: 1–4).

At V0, MVCQD was positively correlated with D4Z4 repeat size ($r = 0.343$, $p < 0.001$). MVCQD was also negatively correlated with the Vignos score ($r = -0.624$, $p < 0.001$), CS and CSS ($r = -0.547$, $p < 0.001$; $r = -0.616$, $p < 0.001$, respectively), and the baseline age-related CSS ($r = -0.618$, $p < 0.001$). Lastly, CSS was positively correlated with the age-related CSS ($r = 0.837$, $p < 0.001$).

3.3. Effect of three years of tailored supplementation on MVC, clinical scores, physical activity, and QoL domains

The linear mixed-effects model evaluated longitudinal changes in MVCQD and MVCQND across four visits (V0–V3) in patients with FSHD taking the tailored antioxidant supplementation (Table 2). Overall, MVCQD increased by 0.83 kg/year ($\beta = 0.83$, $SE = 0.16$, $t = 5.14$, $p < 0.001$), and MVCQND by 1.25 kg/year ($\beta = 1.25$, $SE = 0.16$, $t = 7.83$, $p < 0.001$). The baseline mean MVCQ values were 18.74 kg for the dominant quadriceps and 15.49 kg for the non-dominant quadriceps. However, variability was substantial, as indicated by the inter-individual variance of 143.43 and 139.76, and residual variances of 4.45 and 9.75, respectively. A moderate positive correlation ($r = 0.270$) between intercepts and slopes suggested that individuals with higher baseline MVCQ tended to exhibit greater

improvements. Smoking status ($p = 0.404, 0.771$) and diabetes ($p = 0.275, 0.825$) did not significantly influence the MVCQ trajectories.

The attrition analyses did not show any significant differences in baseline demographics or disease severity between patients who dropped out after V1 and those who continued (Table S1). No association between dropout and baseline Vignos scores ($\chi^2 (3, N = 107) = 2.57, p = 0.46$) or prior MVCQ improvement ($\chi^2 = 0.090, p = 0.760$) was detected (Chi-square test), suggesting that attrition was unrelated to the baseline disease severity or treatment response.

The Brooke score decreased by 0.26 units at V1 ($p < 0.01$) and 1.1 units at V3 ($p < 0.001$), and the greatest change was observed at V3 (Table S2). The Vignos score showed moderate variability (random intercept variance = 3.88) and did not change significantly over time ($p > 0.05$). CS and CSS exhibited high individual variability (variance = 11.65 and 4.23, respectively), but remained stable throughout the follow-up ($p > 0.05$).

The Voorrips total scores increased significantly at V1 ($\beta = 1.036, p < 0.001$, Cohen's $d = 0.213$) (Table 3), driven mainly by the increased sports activity at V1 ($\beta = 0.570, p = 0.023$, Cohen's $d = 0.151$) (Table S3). Daily household and leisure activities remained unchanged.

The baseline (V0) SF-36 scores (QoL) showed mild to moderate impairment (mean PCS = 57.7 ± 21.1 ; MCS = 63.5 ± 20.7) (Table 3 and S4). The PCS increased significantly by 3.19 points at V1 (SE = 1.32, $t = 2.42, p = 0.017$, Cohen's $d = 0.163$) and by 3.99 points at V3 (SE = 1.59, $t = 2.51, p = 0.013$, Cohen's $d = 0.175$). This effect was mainly due to the significant gain in physical role score (V1: +10.63 points, SE = 3.42; $t = 3.11, p < 0.01$, Cohen's $d = 0.284$; V3: +8.98 points, SE = 4.10; $t = 2.19, p < 0.001$, Cohen's $d = 0.220$) (Table S4). The residual variability was substantial (SD = 9.16). A positive correlation was observed between MVCQD and PCS at V1 ($r = 0.300, p = 0.008$).

Table 2
Muscle voluntary contraction (MVC) at baseline (V0) and changes from baseline in patients with complete three-year assessment, at one (V1), two (V2) and three (V3) years of supplementation.

Patient subgroups	Quadriceps	V0	V1	V2	V3	Mixed model P value
		V0 mean (SD)	Difference versus V0 mean (SD) %	Difference versus V0 mean (SD) %	Difference versus V0 mean (SD) %	
All	Dominant	18.4 (11.8)	2.1 (3.4) +9.1	3.0 (4.0) +14.1	2.7 (4.5) +13.9	<0.001
	Non-dominant	15.0 (10.6)***	2.7 (4.4) +16.2	3.7 (5.3) +22.2	4.0 (6.1) +24.7	<0.001
Responders	Dominant	21.3 (8.6)	4.4 (3.2) +24.4	4.9 (4.1) +26.2	4.7 (4.9) +28.8	<0.001
	Non-dominant	18.8 (9.0)	4.5 (4.7) +28.2	5.5 (5.2) +30.1	5.6 (6) +34.3	<0.001
Non-responders	Dominant	13.6 (12.3)***	-0.52 (0.7) -5.6	-0.62 (0.9) -5.2	-1.21 (1.7) -6.8	<0.01
	Non-dominant	10.0 (9.3)***	0.15 (2) +5.0	-0.76 (2.8) +1.9	-0.87 (3.4) -1.5	>0.05

Low MVC (≤ 10 kg)	Dominant	4.9 (2.5)	-0.2 (0.8)	0.1 (1.4)	-0.1 (1.2)	>0.05
	Non dominant	3.5 (2.2)***	-7.9 0.3 (1.3)	-1.2 0.8 (1.7)	-4.8 0.6 (2.0)	>0.05
Medium MVC (10 -20 kg)	Dominant	14.5 (2.9)	+8.4 3.4 (4.0)	+23.5 3.6 (4.5)	+19.1 4.3 (4.9)	0.002
	Non dominant	11.4 (4.3)***	+22.9 2.8 (3.8)	+24.8 3.1 (4.6)	+29.3 3.9 (5.3)	<0.001
High MVC (≥ 20 kg)	Dominant	30.1 (7.8)	+21.6 2.5 (3.2)	+24.3 3.5 (3.9)	+31.2 2.8 (4.5)	<0.001
	Non dominant	25.3 (7.3)***	+9.0 4.2 (5.4)	+12.5 5.2 (6.2)	+10.3 5.3 (7.1)	<0.001
Men	Dominant	21. (13.3)	+16.8 2.5 (3.7)	+20.8 3.5 (4.4)	+22.4 2.7 (4.0)	<0.01
	Non-dominant	16.9 (11.9)***	+10.5 2.9 (5.0)	+16.6 4.1 (6.1)	+14.3 4.0 (6.6)	0.04
Women	Dominant	15.2 (8.8)	+15.9 1.6 (2.9)	+23.0 2.2 (3.3)	+23.9 2.8 (4.5)	<0.001
	Non-dominant	12.7 (8.2)***	+7.5 2.4 (3.5)	+10.6 3.2 (4.1)	+13.3 4.0 (5.3)	<0.001
<50 year old	Dominant	19.1 (12.1)	+16.5 2.6 (3.8)	+21.7 3.6 (4.5)	+26.0 3.9 (4.9)	0.049
	Non-dominant	15.6 (11.4)***	+9.9 3.6 (5.0)	+15.4 5.0 (5.9)	+18.4 6.0 (6.3)	0.007
>50 years old	Dominant	17.7 (11.5)	+18.1 1.6 (2.9)	+26.2 2.3 (3.3)	+33.6 1.6 (11.7)	<0.001
	Non-dominant	14.4 (9.7)***	+8.3 1.8 (3.4)	+12.8 2.4 (4.4)	+24.2 2.1 (10.2)	0.007
			+14.1	+18.5	+31.4	

Abbreviations: IQR, interquartile range; MVC, Muscle voluntary contraction; N, number of subjects; SD, standard deviation; V0, baseline visit.
* indicates significant difference between dominant and non-dominant quadriceps (paired *t*-test).

Table 3

Voorrips score and health-related QoL scores in the different patient groups. P values are based on a linear mixed model.

	All patients				
	V0 mean (SD)	One-year group Difference V1-V0 mean (SD)	P value Mixed Model	Three-year group Difference V3-V0 mean (SD)	P value Mixed Model
Voorrips activity score					
All	5.53 (4.14)	1.04 (2.81)	<0.001	1.55 (3.2)	0.060
Low MVC _{QD}	4.39 (2.77)	0.59 (2.55)	0.252	1.51 (2.88)	0.734
Medium MVC _{QD}	5.10 (4.73)	1.34 (2.43)	0.007	2.15 (3.25)	0.430
High MVC _{QD}	6.57 (4.27)	1.11 (3.21)	0.030	1.19 (3.22)	0.169
Men	6.19 (4.74)	1.52 (3.21)	0.057	2.15 (3.65)	0.043
Women	4.71 (3.09)	0.43 (2.09)	0.306	0.78 (2.18)	0.951
<50 years of age	5.81 (3.90)	0.96 (2.99)	0.030	1.43 (3.47)	0.169
>50 years of age	5.31 (4.42)	0.88 (3.05)	0.054	7.43 (5.89)	0.127
SF-36 physical component score (PCS)					
All	57.7 (21.1)	3.2 (10.7)	0.017	3.7 (11.4)	0.013

Low MVC _{QD}	47.0 (21.4)	5.3 (8.7)	0.051	5.6 (12.3)	0.094
Medium MVC _{QD}	55.0 (22.9)	6.9 (9.5)	0.005	5.7 (7.5)	0.060
High MVC _{QD}	65.8 (16.5)	-1.1 (10.3))	0.922	1.9 (12.8)	0.416
Men	60.9 (20.5)	3.5 (12.0)	0.057	5.3 (11.9)	0.021
Women	53.6 (21.5)	2.8 (8.7)	0.157	1.5 (10.5)	0.289
<50 years of age	64.9 (19.4)	2.4 (11.1)	0.179	2.4 (12.7)	0.064
>50 years of age	50.6 (20.5)	4.0 (10.2)	0.049	4.2 (14.1)	0.087
SF-36 mental component score (MCS)					
All	63.5 (20.7)	1.5 (12.3)	0.340	3.0 (17.6)	0.056
Low MVC _{QD}	58.2 (25.0)	2.2 (14.0)	0.549	6.8 (21.8)	0.090
Medium MVC _{QD}	61.4 (19.9)	4.1 (9.2)	0.206	6.7 (17.1)	0.105
High MVC _{QD}	67.9 (18.1)	-1.0 (13.1)	0.810	0.1 (17.3)	0.832
Men	65.5 (18.5)	1.7 (11.7)	0.457	3.6 (17.1)	0.165
Women	60.8 (23.3)	1.3 (13.2)	0.634	2.2 (18.7)	0.176
<50 years of age	66.8 (19.3)	2.1 (12.5)	0.349	1.1 (17.0)	0.188
>50 years of age	60.2 (21.9)	0.9 (12.3)	0.730	4.1 (15.7)	0.161

Overall, these findings demonstrate sustained improvements in muscle strength, clinical function and physical activity over the three years of tailored anti-oxidant supplementation.

3.3.1. Strength–function trajectories and responder phenotypes in FSHD1

To assess the clinical relevance of MVC_{QD} (+0.83 kg/year) and MVC_{QND} (+1.25 kg/year) increase during the treatment, distribution-based surrogate estimates (X1 and MDC95) were used to determine two MVC_{QD} threshold values to identify responders (0.374 kg and 0.733 kg). ROC analyses showed high discrimination (AUC = 0.982–0.983, respectively) when using the threshold of 0.370 kg, with high sensitivity and specificity (1 and 0.656). Participants with MVC_{QD} values above these thresholds were classified as responders.

3.3.2. Baseline characteristics and early trajectories of response (crosssectional and correlation analyses)

Then, the baseline demographic, clinical, and functional characteristics were compared in patients classified as responders and nonresponders using the previously identified MVC thresholds (Table 4).

Using the 0.374 kg threshold, responders ($n = 79$) showed significantly higher baseline MVCQD and MVCQND scores ($p < 0.001$, Cohen's $d = 1.27$ and 0.97 , respectively) (Table 4), lower Vignos scores ($p = 0.001$, Cohen's $d = 0.85$) and better clinical scores (CS, CSS) ($p = 0.001$; Cohen's $d = 0.76$ and 0.98 , respectively) compared with non-responders (Table 4). D4Z4 repeat length, Brooke score, age or levels of vitamins E and C, or trace elements (Cu, Zn, Se) were not different between groups.

MVCQD was negatively correlated with the Vignos score and CSS in responders ($r = -0.549$, $p < 0.001$; $r = -0.553$, $p < 0.001$, respectively) and also non-responders ($r = -0.554$, $p = 0.002$; $r = -0.501$, $p = 0.006$, with CSS), suggesting that higher initial muscle strength is linked to less severe disease at baseline.

At V1, in responders, MVCQD ($p < 0.001$, Cohen's $d = 0.503$) and MVCQND ($p < 0.001$, Cohen's $d = 0.459$) (Table 2) were significantly increased and the Brooke score was decreased ($p < 0.001$; Cohen's $d = 0.442$), indicating functional gains (Table S2). Conversely, in nonresponders MVCQD was significantly decreased ($p < 0.001$, Cohen's $d = 0.042$), without changes in MVCQND and Brooke score (Table 2 and S2). CS remained stable in responders, but increased in non-responders ($p = 0.002$, Cohen's $d = 0.228$). The differences in changes between groups for MVCQD, Brooke score, and CS were statistically significant ($p < 0.001$, Cohen's $d = 0.769$; $p = 0.04$, Cohen's $d = 0.723$; $p < 0.001$, Cohen's $d = 1.74$, respectively).

Higher baseline MVCQD was associated with greater improvements at V1 ($r = 0.347$, $p = 0.007$), and higher baseline CS was associated with less improvement ($r = -0.400$, $p = 0.002$) (Table S5).

Similar patterns were observed using the higher MVCQD cutoff of 0.733 kg. In responders ($n = 75$) MVCQD increased at V1 ($p < 0.001$, Cohen's $d = 0.503$), whereas it decreased in non-responders ($p = 0.001$, Cohen's $d = 0.042$) (Table S2). The differences in delta MVCQD was significant ($p < 0.001$, Cohen's $d = 1.769$).

Overall, responders had better baseline muscle strength and clinical scores than non-responders, without differences in age, sex, or vitamin levels. Higher initial muscle strength was linked to less severe disease. After one year of antioxidant supplementation, muscle strength and clinical function improved in responders, and decreased in nonresponders. The baseline muscle strength and clinical scores predicted the treatment response.

3.3.3. Longitudinal outcomes

Using linear mixed-effects models, the trajectories of clinical and functional scores were compared between responders and nonresponders based on the two MVCQD thresholds (0.374 kg and 0.734 kg). Using the 0.374 kg cutoff, in responders, MVCQD increased by 1.64 kg per year ($\beta = 1.64$, $SE = 0.18$, $t = 9.11$, $p < 0.001$) and MVCQND increased by 2.01 kg per

year ($\beta = 2.01$, $SE = 0.23$, $t = 8.75$, $p < 0.001$) (Table 2). Conversely, in non-responders, MVCQD decreased by 0.41 kg per year ($\beta = -0.41$, $SE = 0.09$, $t = -4.35$, $p < 0.001$) without changes in MVCQND ($\beta = -0.20$, $SE = 0.17$, $t = -1.16$, $p > 0.1$) (Table 2). The random effects analysis indicated significant variability among subjects, with a variance component of 153.09 for the intercept and 0.71 for the residuals.

Using the 0.374 kg cutoff, in responders, the Brooke score decreased over time ($\beta = -0.260$ at V1, $t = -2.91$, $p < 0.01$; $\beta = -0.460$ at V3, $t = -4.50$, $p < 0.001$) (Table S2), indicating functional improvement. In non-responders, the scores remained stable. The interaction between time and group was significant ($p = 0.001$ at V1; $p = 0.020$ at V3), suggesting a slower rate of improvement in non-responders. The Vignos score remained stable in responders, and tended to worsen in nonresponders at V3 ($\beta = 0.330$, $p = 0.05$ – 0.06). Clinical status (CS) remained stable in responders, but increased significantly in non-responders at V1 and V3 (mean increases of 0.793 and 0.804; $p < 0.01$) (Table S2). The interaction between time and group was significant ($p < 0.001$), confirming the different disease trajectories between groups.

Similar patterns were observed with the MVCQD cutoff of 0.734 kg. In responders, MVCQD increased by 1.64 kg per year ($\beta = 1.64$, $SE = 0.19$, $t = 8.88$, $p < 0.001$), and MVCQND by 2.00 kg per year ($\beta = 2.00$, $SE = 0.24$, $t = 8.42$, $p < 0.001$) (Table S2). Conversely, in non-responders, MVCQD decreased over time ($\beta = -0.19$, $SE = 0.09$, $t = -2.11$, $p < 0.05$), but not MVCQND ($\beta = 0.061$, $SE = 0.195$, $t = 0.32$, $p > 0.1$) (Table S2).

The Brooke score improved over time in responders ($\beta = -0.260$ at V1, $p < 0.01$; $\beta = -0.460$ at V3, $p < 0.001$) and remained relatively stable in non-responders (Table S2). The improvement rate was lower in non-responders, as indicated by significant time-by-group interactions ($\beta = 0.563$, $t = 3.281$, $p = 0.001$ at V1; $\beta = 0.432$, $t = 2.324$, $p = 0.020$ at V3). Moreover, in non-responders, CS increased at both V1 and V3 (0.700 and 0.949 $p < 0.01$, respectively) (Table S2), indicating disease progression.

Overall, after three years of antioxidant supplementation, significant improvements in functional and clinical outcomes were observed only in responders (using both MVCQD thresholds).

3.4. Effect of baseline MVCQD on outcomes

Based on the finding that baseline clinical and functional factors were associated with the response to treatment and disease progression, the impact of baseline quadriceps muscle strength on outcomes was thoroughly investigated. For this, patients were categorized into three groups based of their baseline MVCQD: low (≤ 10 kg, $N = 28$), medium (10–20 kg, $N = 35$), and high (≥ 20 kg, $N = 44$). At baseline, the MVCQD and MVCQND scores were higher in the high group (Fig. 2, Table 2) than in the low and the medium MVCQD groups (MVCQD: $\beta = -23.69$, $SE = 1.50$, $p < 0.001$ and $\beta = -10.73$, $SE = 1.18$, $p < 0.001$; MVCQND: $\beta = -20.67$, $SE = 1.50$, $p < 0.001$ and $\beta = -10.20$, $SE = 1.18$, $p < 0.001$, respectively).

Table 4
Baseline demographic and clinical characteristics of responders and non-responders.

	Threshold delta MVC _{QD} 0.374				Threshold delta MVC _{QD} 0.733		
	Whole population	Responders n = 79	Non-responders n = 28	Between-group difference	Responders n = 75	Non-responders n = 32	Between-group difference
	V0 mean (SD)	V0 mean (SD)	V0 mean (SD)	P value Cohen's d	V0 mean (SD)	V0 mean (SD)	P value Cohen's d
Men - Women, n		46 - 33	13 - 15		44 - 31	15 - 17	
Age (years)	47.0 (16.1)	45.4 (16.2)	48.6 (16.9)	0.391	45.0 (17.3)	48.9 (16.4)	0.268
D4Z4 repeat number, mean (SD)	5.9 (2.1)	6.0 (1.9)	5.7 (1.7)	0.195	5.9 (2.0)	5.8 (1.6)	0.244
MVC _Q , kg				0.403			0.748
Dominant	18.4 (11.8)	21.3 (8.6)	13.6 (12.2)	0.219	21.5 (8.8)	14.1 (12.0)***	0.055
Non dominant	15.0 (10.6)	18.8 (9.0)	10.0 (9.3)	<0.001	19.0 (9.0)	10.4 (9.2)***	<0.001
Brooke score	2.7 (0.8)	2.6 (0.9)	2.8 (0.7)	1.270	2.7 (0.9)	2.7 (0.7)	0.751
Vignos score	2.3 (1.9)	1.8 (1.5)	3.3 (2.4)	0.442	1.8 (1.5)	3.2 (2.3)	0.949
Clinical score (CS)	5.7 (3.3)	4.9 (3.1)	7.3 (3.4)	0.234	4.8 (3.1)	7.2 (3.3)	0.638
Clinical Severity Score (CSS)	2.7 (2)	2.1 (1.6)	3.9 (2.0)	0.001	2.1 (1.6)	3.8 (2.3)	0.0
Age-corrected CSS	121.7 (85.6)	97.8 (65.4)	170.3 (101.4)	0.755	96.1 (67.3)	166.5 (97.2)	0.776
				0.979			0.927
				0.850			0.915

Abbreviations: N, number of subjects; SD, standard deviation; MVC_Q, quadriceps muscle voluntary contraction.

Longitudinal analyses using a linear mixed-effects model revealed significant main effects of time and group on both MVCQD and MVCQND ($p < 0.001$, $p < 0.001$, respectively), indicating overall changes in muscle strength over time and between groups (Table 2). The interaction between time and group also was significant ($p = 0.049$ for MVCQD, $p = 0.002$ for MVCQND), suggesting that muscle strength change rate over time differed between groups. These findings remained statistically robust after FDR correction (MVCQD: adjusted p-values: time = $1.32e-07$, groups = $6.60e-16$, interaction = $2.22e-04$; MVCQND: adjusted p-values: time = $8.58e-09$, groups = $6.60e-16$, interaction = $2.04e-03$).

For the dominant quadriceps, strength increased significantly over time in the high MVCQD group (reference group) ($\beta = 0.91$, SE = 0.19, $p < 0.001$) (Table 2) as well as in the medium MVCQD group ($\beta = 0.58$, SE = 0.29, $p = 0.040$), and to a lower extent, in the low MVCQD group ($\beta = -0.99$, SE = 0.36, $p = 0.005$). Similarly, for the non-dominant quadriceps, a significant main effect of time was observed ($\beta = 1.7$, SE = 0.22, $p < 0.001$) for the high and medium MVCQD groups, and to a lower extent in the low MVCQD group ($\beta = -1.57$, SE = 0.36, $p = 0.005$).

Regarding physical activity, daily household activity levels remained stable in the three groups across visits (Table 3, Table S6), although the daily household activity scores were lower in the low MVCQD group than the high MVCQD group (estimate = -0.425, $p < 0.001$) (Table S6). Sports activities increased at V1 and V3 compared with baseline (V1: estimate = 0.570, $p = 0.024$; V3: estimate = 1.255, $p = 0.027$). However, participation in sports activities was lower in the low and medium MVCQD than the high MVCQD group (low: estimate = -2.631, $p = 0.001$; medium: estimate = -1.627, $p = 0.025$). No significant change was observed in leisure activities over time (Table S6). The Voorrips total score increased significantly in the high and medium MVCQD groups at V1 compared with baseline ($\beta = 1.11$, $p = 0.014$, Cohen's d = 0.213; $\beta = 1.34$, $p = 0.007$, Cohen's d = 0.254, respectively), but not at V3 (Table 3, Table S6).

Physical functioning scores were significantly lower at baseline in the low (-40.73, $p < 0.001$) and medium (-19.57, $p = 0.005$) MVCQD groups (Table S7). The mixed-effects models showed significant improvements over time in several QoL domains (SF-36) (Table 3

and S7). The physical role score in the low MVCQD group increased significantly from baseline to V3 ($\beta = 3.5$, $p < 0.001$), but remained lower than in the high MVCQD group ($p = 0.046$). The SF-36 PCS increased in the medium MVCQD group (+6.9 points, $p = 0.005$, Cohen's $d = 0.208$ at V1, +5.7 points, $p = 0.060$, Cohen's $d = 0.392$ at V3), and bodily pain improved at V1 (+6.4 points, $p = 0.050$, Cohen's $d = 0.282$) and general health at V3 (+8.6 points, $p = 0.008$, Cohen's $d = 0.529$) (Table S7). The vitality and mental health scores improved in the medium MVCQD group at V3 (+6.8 points, $p = 0.022$, Cohen's $d = 0.502$; +6.9 points, $p = 0.033$, Cohen's $d = 0.485$, respectively).

Overall, patients with higher baseline quadriceps strength showed greater improvements over three years of tailored antioxidant supplementation, with significant gains in muscle strength, physical activity and QoL outcomes. These effects were moderate in the medium MVCQD group and limited in the low MVCQD group. Therefore, the baseline quadriceps strength predicted the functional and quality of life outcomes.

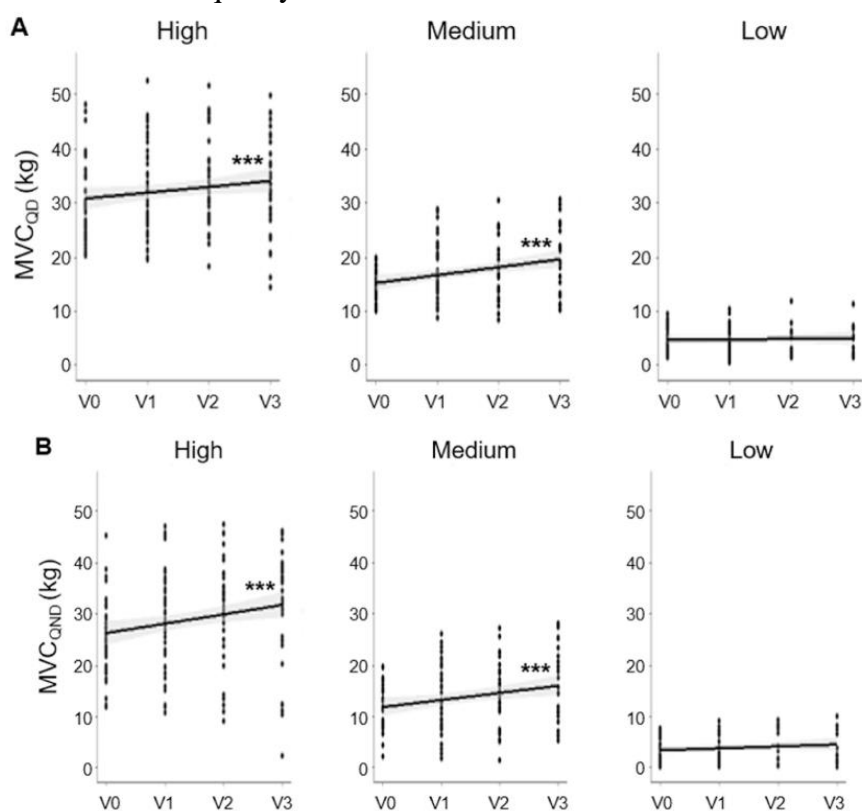


Fig. 2. Quadriceps maximal voluntary contraction (MVC) in patients with FSHD at baseline (V0) and after one to three years of personalized antioxidant supplementation.

Scatterplots with linear trend and confidence intervals based on a linear mixed model for MVC in dominant (A) and non-dominant (B) quadriceps in the High, Medium and Low MVC_{QD} groups at baseline (V0) and at the follow-up visits at year 1 (V1), 2 (V2) and 3 (V3) during the personalized antioxidant supplementation (Low: $n = 28$ at V0 and V1, $n = 13$ at V2 and V3; medium: $n = 35$ at V0 and V1, $n = 26$ at V2 and V3, high: $n = 44$ at V0 and V1, $n = 35$ at V2 and V3). *** p -value < 0.001 (mixed model).

3.5. Sex differences in outcomes

At baseline, muscle strength was higher in men than women: by 6.64 kg for MVCQD ($t = 2.84$, $p < 0.001$) and by 4.71 kg for MVCQND ($t = 2.05$, $p = 0.04$) (Fig. 3, Table 2). During the three-year of treatment, MVCQD ($\beta = 0.93$, $p < 0.001$) and MVCND ($\beta = 1.21$, $p < 0.001$) significantly increased in both sexes. The interaction between sex and time was not

significant (MVCQD: $p = 0.770$; MVCQND: $p = 0.840$), indicating similar rates of strength improvement in both sexes over the study period.

At baseline, the daily household activity scores were higher in women (1.68, $p < 0.001$) than men (- 0.281, SE = 0.102, $p = 0.006$) (Table S3). During the follow-up, in women, the daily household activity scores significantly decreased (- 0.11, $p = 0.009$, Cohen's $d = 0.271$ at V3) and the sports activities scores increased only at V3 (+0.88 points, $p = 0.025$, Cohen's $d = 0.319$). Conversely, in men, the sports activities scores increased over time (V1: +0.92 points, $p = 0.030$, Cohen's $d = 0.203$, V3: +1.53 points, $p < 0.004$, Cohen's $d = 0.337$) (Table S3) as well as the total activity score (+1.52 points, $p = 0.001$, Cohen's $d = 0.269$ at V1; +2.15 points, $p = 0.019$, Cohen's $d = 0.394$ at V3), with a significant sex-by-time interaction ($p = 0.043$) (Table 3, Table S3).

Physical role scores increased similarly in both sexes (men: +10.6 points, $p = 0.029$, Cohen's $d = 0.297$; women: +10.7 points, $p = 0.041$, Cohen's $d = 0.227$, respectively) (Table S4), whereas the bodily pain score increased only in men (+5.7 points, $p = 0.034$, Cohen's $d = 0.253$ at V1; +7.9 points, $p = 0.011$, Cohen's $d = 0.349$ at V3). The SF-36 PCS improved in men (+3.5 points, $p = 0.057$, Cohen's $d = 0.182$ at V1 and +5.3 points, $p = 0.021$, Cohen's $d = 0.280$ at V3) (Table 3 and S4) as well as the SF-36 vitality score at V3 (+5.5 points, $p = 0.024$, Cohen's $d = 0.445$) (Table S4). Women showed significant gains in general health (+5.7 points, $p = 0.024$, Cohen's $d = 0.330$) and mental health at V3 (+4.8 points, $p = 0.040$, Cohen's $d = 0.309$).

Overall, men started with higher baseline strength and activity levels, but showed similar rates of strength improvement over time as women. Women reduced household activity, but increased sports activities, while men showed total activity scores improvements. Physical role scores improved in both sexes, whereas bodily pain and vitality improved in men, and health and mental health in women.

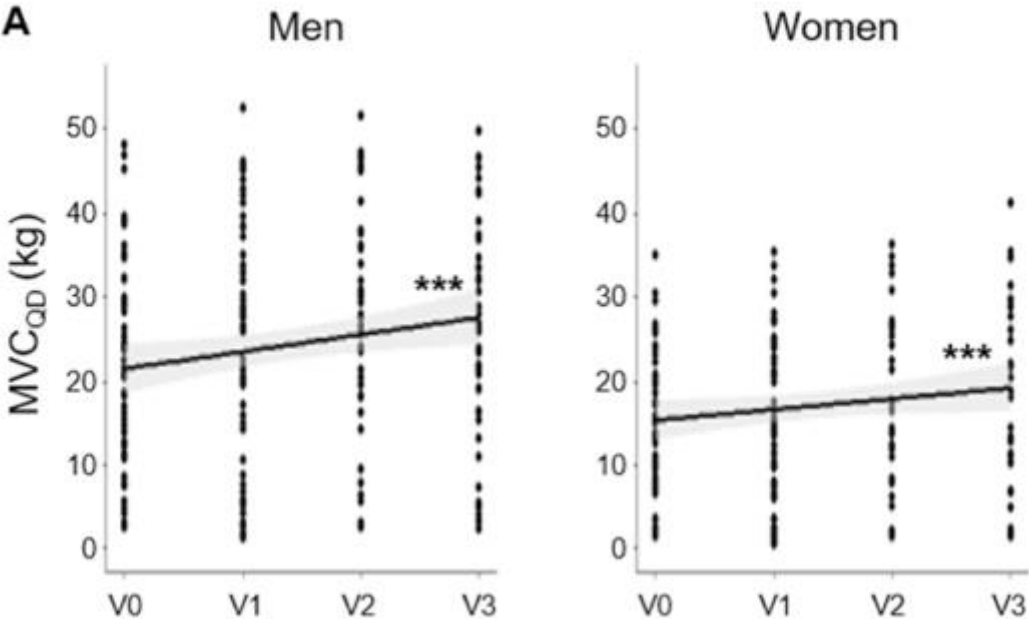
3.6. Age difference in outcomes

The linear mixed-effects model indicated that age alone did not significantly influence the MVCQD scores at baseline (MVCQD: $p = 0.510$, and MVCQND: $p > 0.05$). However, a significant interaction between age and time was observed (MVCQD: $\beta = - 0.023$, SE = 0.008, $p = 0.004$ and MVCQND: $\beta = - 0.024$, SE = 0.010, $p = 0.016$), suggesting that the magnitude of improvement over time was smaller in older patients. When comparing <50-year-old patients ($n = 55$ at V1 and $n = 32$ at V3) and ≥ 50 -year-old patients ($n = 52$ at V1 and $n = 42$ at V3), the annual increases in MVCQD and MVCQND was higher in the younger group (1.19 kg, $p = 0.049$ and 1.77 kg, $p < 0.001$, per year respectively) than in the older group (0.68 kg, $p = 0.00016$ and 0.89 kg, $p = 0.007$, per year, respectively) (Table 2). After adjusting for multiple comparisons, these differences were no longer statistically significant (adjusted $p = 0.073$). The relationship between time and MVC was similar across age groups.

Moreover, household activity scores were higher in <50-year-old patients (+0.47 points per year, $p < 0.001$) compared with older patients (Table S3). Conversely, the sport and total activity scores were not significantly different between age groups (Table 3, Table S3).

Regarding QoL, younger patients exhibited higher PCS scores (+11.64, $p = 0.001$) and the difference between age groups remained stable during the follow-up (Table 3, Table S8).

Overall, age did not significantly affect baseline muscle strength, but ≥ 50 -year-old patients showed smaller improvements over time. Younger individuals had higher household activity and quality of life scores, particularly in physical health, and maintained these advantages throughout the follow-up period.



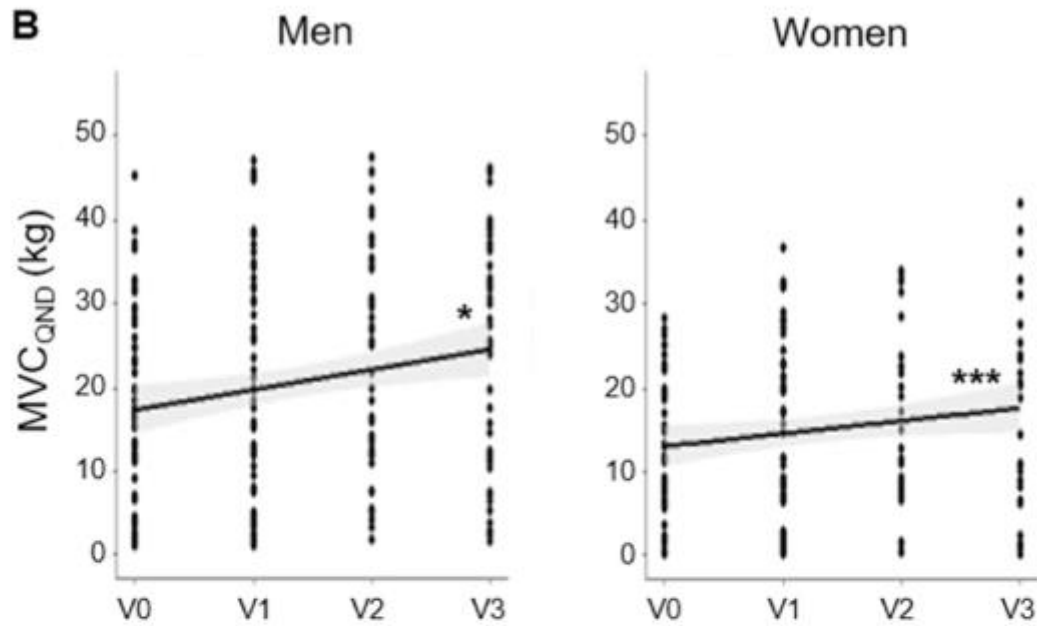


Fig. 3. Quadriceps maximal voluntary contraction (MVC) in patients with FSHD at baseline (V0) and after one to three years of personalized antioxidant supplementation.

Scatterplots with linear trend and confidence intervals based on a linear mixed model for MVC in dominant (A) and non-dominant quadriceps (B) in men (N = 59 for V0 and V1, N = 44 for V2 and V3) and women (N = 48 for V0 and V1, N = 30 for V2 and V3) at baseline (V0) and at the follow-up visits at year 1 (V1), 2 (V2) and 3 (V3) during personalized antioxidant supplementation. *** p-value <0.001 and * p < 0.05 (Mixed model).

3.7. Four- and five-year follow-up groups

A linear mixed-effects model with random intercepts for subjects showed that MVCQD increased significantly by 0.57 kg per year (SE = 0.10, $p < 0.001$), and MVCQND by 0.87 kg per year ($p < 0.001$) over the 5 years of follow-up. Despite individual variability, these upward trajectories persisted across the cohort.

To assess potential bias, the baseline demographic and clinical characteristics of patients who received tailored antioxidant supplementation at V3, V4 and V5 and of patients who dropped out after V3 or V4 were compared (Table S1). No significant difference was observed between the V4 group and dropouts after V3, or between the V5 group and dropouts after V4. No significant association between dropout status and disease severity, assessed using the Vignos scale scores, was detected (chi-square = 0.780, $p > 0.05$ after V3; chi-square = 0.824, $p > 0.05$ after V4).

Similarly, the proportion of patients with MVCQD improvement in the previous year was comparable between dropouts and continuers (41% of dropouts vs 51% of continuers). Prior MVCQD improvement was not associated with dropout (chi-square = 0.780, $p > 0.05$ after V3; chisquare = 0.156, $p > 0.05$ after V4). These findings suggest that attrition was unrelated

to baseline disease severity or treatment response, reducing the potential bias in the study outcomes.

Overall, MVCQ strength increased significantly over the five years of tailored antioxidant supplementation, with consistent upward trends across the cohort. Attrition was unlikely to bias the results related to disease severity or treatment response.

3.8. Patients with temporary supplement interruption

In patients who temporarily interrupted supplementation between V3 and V5, models revealed significant increases in both MVCQD ($\beta = 4.01$, $t(34) = 3.99$, $p < 0.001$) and MVCQND ($\beta = 4.98$, $t(34) = 3.73$, $p = 0.0007$) from V0 to V3. However, after supplementation interruption, MVCQD and MVCQND were not different from baseline (MVCQD: $\beta = 0.78$, $t(34) = 0.78$, $p = 0.44$; MVCQND: $\beta = 2.00$, $t(34) = 1.50$, $p = 0.14$), suggesting that the initial gains observed at V3 were not sustained following the arrest of antioxidant supplementation (Table S9) returning to the baseline levels.

4. Discussion

This study investigated the effect of a patient-tailored antioxidant supplementation on FSHD management in a real-world setting. To account for the variability in oxidative stress markers among patients with FSHD, antioxidant doses were tailored based on the individual blood concentrations of vitamins C and E and some essential trace elements (zinc, copper, and selenium). This routine care allowed us to develop and evaluate a decision-tree model, currently patent-pending, which determines antioxidant supplementation dosages adapted to the patient's profile. This represents a significant step toward personalized therapy in FSHD, emphasizing the importance of adjusting interventions to individual biochemical signatures.

In the present study, 107 patients were monitored for one year and 74 patients were followed for three years. The estimated baseline MVCQ values (18.74 kg for MVCQD and 15.49 kg for MVCQND) reflect the typical reduced muscle function in FSHD. Baseline clinical data showed most patients had mild to moderate disease severity with a median CS of 5, with no patients at zero severity; about 12% had very mild symptoms, and 25% exhibited more advanced impairment. In agreement, the median CSS was 3, indicating mild to moderate severity. Functional assessments revealed that most patients had moderate upper and lower limb impairments. These baseline data offer valuable insights into the overall disease burden within this population. Moreover, the D4Z4 repeat size was negatively correlated with both baseline CS and CSS, indicating that smaller repeat sizes are associated with higher disease severity. Conversely, D4Z4 repeat size was positively correlated with MVCQD. The Brooke and Vignos scores were positively correlated with CS and CSS, reinforcing the relationship between clinical and functional impairments.

The longitudinal analysis using a linear mixed-effects model revealed increases in MVCQ over the three years of antioxidant supplementation, with an average yearly gain of 0.83 kg

per year for MVCQD and 1.25 kg for MVCQND. This model identified a strong predictive value of the baseline muscle strength. Patients classified as responders, using the MVCQD increase threshold of 0.374 kg, showed gains in muscle strength that correlated with improvements in the Brooke score. Conversely, nonresponders showed minimal or negative changes. These findings high light baseline MVCQ is a robust predictor for identifying patients who might benefit from tailored antioxidant supplementation, emphasizing the critical role of early and precise assessment of muscle strength. Moreover, patients with high baseline MVCQD values (≥ 20 kg) tended to respond better and exhibit more consistent improvements. This underscores the importance of early detection and intervention before severe muscle damage, such as fibrosis [40] and/or fatty infiltration [41], that limits the recovery potential and impairs muscle plasticity.

Inter-individual variability was substantial, with standard deviations near 12 kg for dominant and non-dominant quadriceps, reflecting the heterogeneity of the disease presentation and treatment response in patients with FSHD. Importantly, no significant bias was observed regarding baseline disease severity or dropout patterns.

A moderate positive correlation between baseline muscle strength and improvement rate ($r = 0.270$) suggests that patients with high baseline MVCQD retain a greater proportion of functional muscle fibers and respond better to antioxidant treatment. These findings are consistent with previous studies linking muscle weakness in FSHD to oxidative stress [13,14], reduced oxygen consumption [16], mitochondrial dysfunction [13], reduced capillary density [29], mitochondrial aggregation [13] and increase fat and fibrotic infiltration [17,40,41]. All these alterations contribute to impair muscle quality and contractile performance. Collectively, these findings support the potential of antioxidant treatment to mitigate muscle degeneration in FSHD by targeting both metabolic and structural impairments. Importantly, factors, such as smoking status and diabetes, did not significantly influence the MVCQ trajectories following antioxidant supplementation, indicating that these morbidities may not impact the observed treatment effects within this cohort.

The Voorrips total score, which reflects overall physical activity, showed significant improvements at V1, primarily driven by increased engagement in sport activities. Routine daily activities, including household and leisure pursuits, remained relatively stable throughout the study period. This pattern may reflect a selective increase in higher-intensity or structured physical activities, which could contribute to the observed strength and QoL improvements.

In terms of QoL, assessed with the SF-36, the physical component score increased over time, driven by improvements in the physical role and vitality domains. These results suggest that increased engagement in physical activity might contribute to improved perceptions of physical function and vitality in patients. The stability or improvement of other domains indicates that targeted interventions could exert specific benefits on certain aspects of QoL. Furthermore, attrition was unlikely to bias the results, because there were no significant differences between patients who dropped out and those who completed the study. Collectively, these findings support the hypothesis that with personalized antioxidant supplementation and activity engagement, patients with FSHD may experience stabilization or improvements in muscle strength and QoL over time. Importantly, a persistent positive correlation between MVCQD and PCS was observed after one year of treatment, suggesting a direct link between improvements in quadriceps strength and overall physical well-being.

The linear mixed-effects model analysis showed that age alone did not significantly influence the baseline muscle strength. However, there was a significant interaction between age and time, revealing that older patients experienced a smaller magnitude of improvement over the study period. Specifically, <50-year-old patients demonstrated greater annual increases in MVCQD and MVCQND compared with older patients, although these differences were no longer significant after adjusting for multiple comparisons. Despite similar trajectories over time between age groups, younger patients had higher household activity scores and maintained higher SF-36 PCS scores throughout the follow-up. These findings suggest that although the initial muscle strength may be comparable across ages, younger patients tend to have better functional and QoL outcomes, possibly due to greater activity levels or other age-related factors. Nonetheless, the overall relationship between age and muscle strength progression appears modest, highlighting the need of more studies on age-specific interventions to optimize outcomes in FSHD.

Sex-based analyses highlighted differences in baseline muscle strength, activity levels, and quality of life outcomes among men and women with FSHD. At baseline, men exhibited significantly higher muscle strength, consistent with known physiological differences in muscle mass and strength between sexes. However, MVCQD and MVCQND increased similarly in both sexes over time. This suggests that both sexes are similarly responsive to antioxidant supplementation. Unlike the MVC estimates, sex-related differences in baseline activity levels and QoL measures were more nuanced. Women reported significantly higher household activities at baseline. Over time, this engagement declined slightly, whereas sport activity increased after 3 year of supplementation. This may reflect changes in routine and personal priorities or improved physical capacity. Men increased participation in sports activities over time, with a significant sex-by-time interaction. In QoL measures, both men and women experienced similar improvements in physical role. However, sex-specific QoL improvements were also observed, such as greater pain reduction and vitality improvement in men and gains in general and mental health in women. This suggests that the perceived and functional impact may be influenced by social roles and health perceptions. These insights underscore the importance of personalized, gender-sensitive approaches in managing FSHD, tailoring interventions to optimize physical and psychosocial outcomes.

Overall, our findings on the real-life effectiveness of antioxidant supplementation in patients with FSHD are encouraging. The data suggest that antioxidant therapy may offer benefits, particularly in improving muscle strength and arm function, for a subset of patients with FSHD1, especially those with higher baseline strength and less advanced disease. The observed stability in the overall disease severity scores aligns with the typically slow FSHD progression, supporting the potential for early intervention. Baseline functional parameters, such as MVCQD, emerged as valuable predictors of the treatment response, underscoring the importance of personalized therapeutic strategies. Early identification of responders could optimize clinical outcomes. Moreover, the divergence in clinical trajectories between responders and non-responders further emphasizes the need to develop predictive biomarkers and tailor interventions accordingly.

Several limitations must be acknowledged. First, the variability in patients' clinical presentation at baseline may have complicated the interpretation of outcomes. Additionally, the limited number of patients in some subgroups, particularly in the low-force group restricts the statistical power and generalizability of the findings. Moreover, QoL is a subjective endpoint that may be biased by individual perceptions and external factors; however, QoL

encompasses more than physical health, including mental well-being and social support. Future interventions should also address these psychological aspects. They should also consider of psychological factors.

The absence of a placebo-controlled design limits our ability to establish definitive evidence of efficacy. Randomized clinical trials with placebo controls are the gold standard for establishing causal relationship and assessing short-term efficacy; however, their long-term feasibility is often limited due to ethical and practical considerations. Conversely, observational studies, which better reflect real-world clinical setting, present challenges in establishing causality and controlling for biases [42]. To address these limitations, a complementary approach that integrates the methodological rigor of randomized clinical trials and the external validity of observational studies is recommended.

Furthermore, our study did not include extensive biomarker analyses, such as oxidative stress markers, natural history data, or advanced imaging, that could have helped to understand the underlying mechanisms and facilitate patient stratification. Although the thresholds used for muscle strength changes are transparent and quantitative, they were based on distribution estimates rather than validated minimal clinically important differences. Future research should focus on establishing FSHD-specific minimal clinically important differences through patient-centered approaches and validated functional endpoints.

It is also important to recognize that gains in muscle strength do not necessarily correspond to meaningful functional improvements. The increases in MVCQD and arm scores suggest a positive trend, but their clinical significance requires further validation. Longer follow-up periods with larger cohorts are essential to determine whether early muscle strength gains translate into sustained functional benefits. Additionally, pre-specified predictors of response should be validated in independent populations, and tissue-based biomarkers, such as high-resolution MRI, force–frequency analysis and biopsies of the *vastus lateralis*, should be integrated to better understand the mechanistic relationship between tissue structure and function.

Regarding the outcome measures, most participants exhibited minimal quadriceps impairment at baseline (i.e. Vignos score = 1). This indicated preserved ambulation, but also limited the sensitivity of the scale to detect short-term changes. Although objective scores (i.e. CSS and Brooke score) did not show significant changes at V1, the increase in MVCQ suggests a directional trend towards improved quadriceps strength and arm function, potentially serving as early indicators of clinical benefit. The correlation between quadriceps strength improvements and functional measures supports the presence of a clinical signal, although some associations did not reach statistical significance due to limited longitudinal data. Although our study did not include a natural history control group, the observed improvements can be meaningfully interpreted in the context of well-established disease trajectories in FSHD [43,44]. Additional support for a treatment-related effect comes from a subgroup of patients who temporarily interrupted supplementation between year 3 and 5. Despite significant gains between V0 and V3, these patients experienced a significant decrease in quadriceps strength, returning to baseline levels after treatment interruption. This suggests that continued antioxidant supplementation may be necessary to maintain the functional improvements and suggests that the observed muscle strength increases during antioxidant supplementation are unlikely to result solely from natural fluctuations.

5. Conclusion

While our results are preliminary, they highlight the potential of antioxidant therapy as part of a personalized management approach for FSHD, particularly when initiated early. Moreover, they suggest that ongoing therapy is essential for maintaining gains, emphasizing the importance of adherence and possibly developing follow-up protocols to optimize patient outcomes. Future studies should incorporate comprehensive biomarker analyses, validated clinical endpoints, and longer follow-up periods to confirm and expand these findings.

This comprehensive study highlights the potential of personalized antioxidant supplementation in managing FSHD by targeting oxidative stress, a key factor in disease pathology. Customizing antioxidant doses based on individual biomarkers yielded promising improvements in muscle strength, muscle quality, and overall quality of life, underscoring the importance of tailored treatment strategies. The findings also emphasize that early intervention, sustained therapy, and continuous support are vital for maximizing functional gains and preventing disease progression.

CRedit authorship contribution statement

Dalila Laoudj-Chenivresse: Writing – review & editing, Writing – original draft, Visualization, Validation, Supervision, Methodology, Funding acquisition, Formal analysis, Data curation, Conceptualization.

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Marie-Christine Picot: Writing – review & editing, Writing – original draft, Methodology, Formal analysis, Data curation, Conceptualization.

Jean-Paul Cristol: Writing – review & editing, Writing – original draft, Data curation.

Jacques Mercier: Writing – review & editing, Writing – original draft, Supervision, Resources, Investigation, Conceptualization.

Florence Portet: Writing – review & editing, Writing – original draft, Investigation, Data curation.

Data availability statement

As the participants only gave consent to report the summary of data, no individual data can be shared publicly.

Declaration of generative AI and AI-assisted technologies in the writing process

During the preparation of this work the authors did not use AI and AI-assisted technologies.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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References

- [1] J.C.W. Deenen, et al., Population-based incidence and prevalence of facioscapulohumeral dystrophy, *Neurology* 83 (2014) 1056–1059.
- [2] C. Guien, et al., The French national registry of patients with facioscapulohumeral muscular dystrophy, *Orphanet J. Rare Dis.* 13 (2018) 218.
- [3] T. Schatzl, L. Kaiser, H.-P. Deigner, Facioscapulohumeral muscular dystrophy: genetics, gene activation and downstream signalling with regard to recent therapeutic approaches: an update, *Orphanet J. Rare Dis.* 16 (2021) 129.
- [4] M. Dixit, et al., DUX4, a candidate gene of facioscapulohumeral muscular dystrophy, encodes a transcriptional activator of PITX1, *Proc. Natl. Acad. Sci. U. S. A.* 104 (2007) 18157–18162.
- [5] C. Vanderplanck, et al., The FSHD atrophic myotube phenotype is caused by DUX4 expression, *PLoS One* 6 (2011) e26820.
- [6] L.N. Geng, et al., DUX4 activates germline genes, retroelements, and immune mediators: implications for facioscapulohumeral dystrophy, *Dev. Cell* 22 (2012) 38–51.
- [7] V. Sharma, N. Harafuji, A. Belayew, Y.W. Chen, DUX4 differentially regulates transcriptomes of human rhabdomyosarcoma and mouse C2C12 cells, *PLoS One* 8 (2013) e64691.
- [8] G. Andersen, K.P. Prahm, J.R. Dahlqvist, G. Citirak, J. Vissing, Aerobic training and postexercise protein in facioscapulohumeral muscular dystrophy: RCT study, *Neurology* 85 (2015) 396–403.
- [9] D.B. Olsen, M.C. Ørngreen, J. Vissing, Aerobic training improves exercise performance in facioscapulohumeral muscular dystrophy, *Neurology* 64 (2005) 1064–1066.

- [10] Q. Xie, G. Ma, Y. Song, Therapeutic strategy and clinical path of facioscapulohumeral muscular dystrophy: review of the current literature, *Appl. Sci.* 14 (2024) 8222.
- [11] V. Macaione, et al., RAGE-NF- κ B pathway activation in response to oxidative stress in facioscapulohumeral muscular dystrophy, *Acta Neurol. Scand.* 115 (2007) 115–121.
- [12] D. Laoudj-Chenivesse, et al., Increased levels of adenine nucleotide translocator 1 protein and response to oxidative stress are early events in facioscapulohumeral muscular dystrophy muscle, *J. Mol. Med.* 83 (2005) 216–224.
- [13] A. Turki, et al., Functional muscle impairment in facioscapulohumeral muscular dystrophy is correlated with oxidative stress and mitochondrial dysfunction, *Free Radic. Biol. Med.* 53 (2012) 1068–1079.
- [14] E. Passerieux, et al., Effects of vitamin C, vitamin E, zinc gluconate, and selenomethionine supplementation on muscle function and oxidative stress biomarkers in patients with facioscapulohumeral dystrophy: a double-blind randomized controlled clinical trial, *Free Radic. Biol. Med.* 81 (2015) 158–169.
- [15] M. Barro, et al., Myoblasts from affected and non-affected FSHD muscles exhibit morphological differentiation defects, *J. Cell Mol. Med.* 14 (2010) 275–289.
- [16] V.D. Wilson, et al., Impaired oxygen demand during exercise is related to oxidative stress and muscle function in facioscapulohumeral muscular dystrophy, *JCSM Rapid Communications* 1 (2018) 1–13.
- [17] V.D. Wilson, et al., Muscle strength, quantity and quality and muscle fat quantity and their association with oxidative stress in patients with facioscapulohumeral muscular dystrophy: effect of antioxidant supplementation, *Free Radic. Biol. Med.* 219 (2024) 112–126.
- [18] S.T. Winokur, et al., Facioscapulohumeral muscular dystrophy (FSHD) myoblasts demonstrate increased susceptibility to oxidative stress, *Neuromuscul. Disord.* 13 (2003) 322–333.
- [19] L. Hubregtse, et al., An up-to-date myopathologic characterisation of facioscapulohumeral muscular dystrophy type 1 muscle biopsies shows sarcolemmal complement membrane attack complex deposits and increased skeletal muscle regeneration, *Neuromuscul. Disord.* 36 (2024) 6–15.
- [20] A. Karpukhina, et al., Analysis of genes regulated by DUX4 via oxidative stress reveals potential therapeutic targets for treatment of facioscapulohumeral dystrophy, *Redox Biol.* 43 (2021) 102008.
- [21] M. Sasaki-Honda, et al., A patient-derived iPSC model revealed oxidative stress increases facioscapulohumeral muscular dystrophy-causative *DUX4*, *Hum. Mol. Genet.* 27 (2018) 4024–4035.
- [22] R.J. Hillstrom, A.K. Yacapin-Ammons, S.M. Lynch, Vitamin C inhibits lipid oxidation in human HDL, *J. Nutr.* 133 (2003) 3047–3051.

- [23] J.A. Awad, J.D. Morrow, K.E. Hill, L.J. Roberts, R.F. Burk, Detection and localization of lipid peroxidation in selenium- and vitamin E-deficient rats using F2-isoprostanes, *J. Nutr.* 124 (1994) 810–816.
- [24] E. Niki, Antioxidants in relation to lipid peroxidation, *Chem. Phys. Lipids* 44 (1987) 227–253.
- [25] A. Carr, B. Frei, Does vitamin C act as a pro-oxidant under physiological conditions? *FASEB J.* 13 (1999) 1007–1024.
- [26] R.A. Sunde, et al., Longitudinal selenium status in healthy British adults: assessment using biochemical and molecular biomarkers, *Br. J. Nutr.* 99 (2008) S37–S47.
- [27] S. Amzali, et al., Nutritional status of patients with facioscapulohumeral muscular dystrophy, *Nutrients* 15 (2023) 1673.
- [28] A. Bast, G.R.M.M. Haenen, The toxicity of antioxidants and their metabolites, *Environ. Toxicol. Pharmacol.* 11 (2002) 251–258.
- [29] J.M. Statland, et al., Muscle pathology grade for facioscapulohumeral muscular dystrophy biopsies, *Muscle Nerve* 52 (2015) 521–526.
- [30] M.H. Brooke, et al., Clinical trial in duchenne dystrophy. I. The design of the protocol, *Muscle Nerve* 4 (1981) 186–197.
- [31] P.J. Vignos Jr., G.E. Spencer Jr., K.C. Archibald, Management of progressive muscular dystrophy of childhood, *JAMA* 184 (1963) 89–96.
- [32] E. Ricci, et al., Progress in the molecular diagnosis of facioscapulohumeral muscular dystrophy and correlation between the number of KpnI repeats at the 4q35 locus and clinical phenotype, *Ann. Neurol.* 45 (1999) 751–757.
- [33] P.G. M.v. Overveld, Interchromosomal repeat array interactions between chromosomes 4 and 10: a model for subtelomeric plasticity, *Hum. Mol. Genet.* 9 (2000) 2879–2884.
- [34] L.E. Voorrips, A.C. Ravelli, P.C. Dongelmans, P. Deurenberg, W.A. Van Staveren, A physical activity questionnaire for the elderly, *Med. Sci. Sports Exerc.* 23 (1991) 974–979.
- [35] J.E. Ware, C.D. Sherbourne, The MOS 36-item short-form health survey (SF-36). I. Conceptual framework and item selection, *Med. Care* 30 (1992) 473–483.
- [36] J. Ware, K. Ma, S.D. Keller, SF-36 Physical and Mental Health Summary Scales: a User's Manual, vol. 8, 1993, pp. 23–28.
- [37] R Core Team, R A Language and Environment for Statistical Computing, R Foundation for Statistical Computing. - References - Scientific Research Publishing, 2025. <https://www.scirp.org/reference/referencespapers?referenceid=3967248>.
- [38] J.N. Hathcock, Vitamins and minerals: efficacy and safety, *Am. J. Clin. Nutr.* 66 (1997) 427–437.

- [39] S. Oldreive, Safe intakes of vitamins and minerals: recommendations from the expert group on vitamins and minerals, *Nutr. Bull.* 28 (2003) 199–202.
- [40] C.R.S. Banerji, D. Henderson, R.N. Tawil, P.S. Zammit, Skeletal muscle regeneration in facioscapulohumeral muscular dystrophy is correlated with pathological severity, *Hum. Mol. Genet.* 29 (2020) 2746–2760.
- [41] B.H. Janssen, et al., Distinct disease phases in muscles of facioscapulohumeral dystrophy patients identified by MR detected fat infiltration, *PLoS One* 9 (2014) e85416.
- [42] S. Monti, V. Grosso, M. Todoerti, R. Caporali, Randomized controlled trials and real-world data: differences and similarities to untangle literature data, *Rheumatology* 57 (2018) vii54–vii58.
- [43] A. Varma, et al., A longitudinal study of disease progression in facioscapulohumeral muscular dystrophy (FSHD), *Muscle Nerve* 69 (2024) 362–367.
- [44] A Prospective, Quantitative study of the natural history of facioscapulohumeral muscular dystrophy (FSHD), *Neurology* 48 (1997) 38–46.